Three new physalins from *Physalis alkekengi* var. *franchetii*

Wan-Xuan XU,^{a,b} Jian-Chao CHEN,^a Jie-Qing LIU,^a Lin ZHOU,^a Yi-Fen WANG,^a and Ming-Hua QIU^{a,b,*}

^aState Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

^bUniversity of Chinese Academy of Sciences, Beijing 100049, China

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Abstract: Three new physalin steroids, physalin III (1), physalin IV (2), 3-*O*-methylphysalin X (3), together with five known physalins (4–8) were isolated from the 80% EtOH extract of calyces of *Physalis alkekengi* var. *franchetii*. The structures of the new compounds were revealed through 1D and 2D NMR and mass spectroscopic studies.

Keywords: *Physalis alkekengi* var. *franchetii*, steroid, physalin, neophysalin

Introduction

The genus of *Physalis* (Solanaceae), including about 120 species, is widely distributed throughout South and North America, which also includes five species, and two variations which also can be found in China¹. The plant *Physalis alkekengi* var. *franchetii*, is used in traditional Chinese medicine², and is chiefly used for the treatment and prevention of tumors, leishmaniasis, sore throat, cough, eczema, hepatitis and urinary problems³. The chemical constituents of *P. alkekengi* var. *franchetii* are mainly composed of alkaloids, flavonoids, sterols, fatty acids, amino acids, and steroids⁴.

Physalins, as one of the characteristic constituents from *P. alkekengi* var. *franchetii*, is a type of steroids with 16,24-cyclo-13,14-*seco*-ergostane skeleton which has been established by X-ray crystallographic analysis^{5–7}. Neophysalins, are another characteristic structure, and are transformed from physalins through an acid-induced benzilic acid-type rearrangement reaction⁸. Until recently, more than 50 physalins and neophysalins have been identified from *P. alkekengi* var. *franchetii*, *P. agulata*, *P. lancifolia* and *P. minima*. Physalins and neophysalins have tremendous biological activities, including anti-tumor⁹, anti-microbial¹⁰, anti-malarial¹¹, immunosuppressive¹², anti-inflammatory^{13,14}, immunomodulatory¹⁵, cytotoxic¹⁶, and trypanocidal^{17,18} effects.

In this study, five physalins (1, 2, 4–6) and three neophysalins (3, 7, 8) were isolated from *P. alkekengi* var. *franchetii*, three of which (1–3) were new. Their chemical structures and their elucidation are described herein.

Results and Discussion

Compound 1 was obtained as a white powder. The molecular

formula of 1 was determined to be C₂₈H₃₂O₁₂ by HREIMS (*m/z* 560.1885, [M]⁺; calcd 560.1894) and NMR data (Table 1). The ¹H NMR spectrum of 1 (in pyridine-*d*₅) showed four methyl signals at δ_H 1.29, 1.47, 1.65, and 2.02, four oxygenated signals at δ_H 4.01 (H-4), 3.54 (H-6), 5.70 (H-7) and 4.73 (H-22), and two olefinic proton signals at δ_H 6.24 (H-2) and 7.19 (H-3). The ¹³C DEPT spectrum of 1 (in pyridine-*d*₅) displayed the presence of twenty-eight carbons, including four methyls, three methylenes, ten methines (two olefinic carbons and four oxymethanes), eleven quaternary carbons (two ketone groups, two lactone groups, four oxygenated carbons, and one ketal carbon). These signals indicated the structural similarity of 1 and physalin J¹⁹. The chemical shift of the ketal carbon at C-14 (δ_C 103.4) of 1 differently from that (δ_C 107.8) of physalin J, together with one more methyl signal and less an oxygenated CH₂ in 1 indicated that the C(27)-O-C(14) ether

*To whom correspondence should be addressed. E-mail: mhchiu@mail.kib.ac.cn

Table 1. ^1H NMR (600 MHz) and ^{13}C DEPT (150 MHz) data of compounds 1–3

No.	physalin III (1) ^a		physalin IV (2) ^a		3-O-methylphysalin X (3) ^b	
	δ_{H} (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)	δ_{C} , type
1		202.9, C		212.3, C		213.8, C
2	6.24, d (9.8)	132.8, CH	3.12, dd (11.7, 5.4); 2.85, dd (11.7, 5.7)	47.1, CH ₂	3.13, dd (13.4, 6.5); 2.62, m	41.2, CH ₂
3	7.19, dd (9.8, 6.5)	145.6, CH	4.67, dd (5.7, 5.4)	67.7, CH	4.42–4.38, m	76.7, CH
4	4.01, d (6.3)	70.3, CH	6.32, d (4.4)	130.5, CH	5.70, d (4.1)	122.4, CH
5		66.2, C		145.8, C		142.8, C
6	3.54, br. s	62.9, CH	4.79, d (3.8)	77.4, CH	6.28, dd (10.4, 1.5)	127.5, CH
7	5.70, br. s	65.4, CH	5.23, dd (3.5, 1.6)	70.6, CH	6.17, dd (10.4, 3.4)	127.9, CH
8	2.93, m	44.1, CH	3.39, dd (11.7, 1.6)	43.8, CH	2.81, m	47.7, CH
9	3.45, m	32.2, CH	2.11, dd (11.7, 2.7)	32.2, CH	2.71–2.74, m	32.8, CH
10		49.8, C		53.3, C		51.0, C
11	2.89, m	20.4, CH ₂	1.63–1.54, m; 2.34, m	23.9, CH ₂	2.05, d (4.4); 1.71–1.59, m	28.0, CH ₂
12	2.47, m; 2.25–2.31, m	32.1, CH ₂	2.58, ddd (16.5, 13.0, 6.2); 1.95, dd (16.5, 9.4)	26.8, CH ₂	1.92, d (1.3); 2.04, m	31.9, CH ₂
13		80.8, C		80.3, C		80.8, C
14		103.4, C		108.5, C		82.3, C
15		217.0, C		209.7, C		169.5, C
16	3.23, s	55.6, CH	3.26, s	55.3, CH	2.60, s	51.1, CH
17		83.6, C		81.9, C		83.9, C
18		173.5, C		173.7, C		173.3, C
19	1.65, s	15.3, CH ₃	1.78, s	19.8, CH ₃	1.36, s	21.2, CH ₃
20		83.9, C		82.6, C		82.5, C
21	2.02, s	22.3, CH ₃	2.32, s	22.9, CH ₃	1.76, s	22.3, CH ₃
22	4.73, br. s	77.6, CH	4.75, m	78.0, CH	4.58, dd (4.4, 1.4)	76.1, CH
23	2.14–2.19, m; 1.92, d (14.9)	27.5, CH ₂	2.11, m	33.1, CH ₂	1.97, m	33.0, CH ₂
24		36.0, C		32.0, C		29.7, C
25	3.42, m	42.4, CH	3.05, d (3.8)	51.2, CH	2.57, dd (11.4, 2.3)	41.7, CH
26		173.0, C		168.1, C		169.4, C
27	1.29, d (7.5)	17.5, CH ₃	4.02, dd (13.4, 3.9); 4.83, dd (13.4, 4.5)	62.4, CH ₂	4.48, dd (12.7, 3.3); 4.32, m	61.7, CH ₂
28	1.47, s	26.4, CH ₃	1.30, s	26.1, CH ₃	1.45, s	31.7, CH ₃
OMe					3.27, s	55.7, CH ₃

^ain pyridine-*d*₅, ^bin CDCl₃.

bridge was ruptured. This was further determined by the HMBC correlations (Figure 1) from Me-27 (δ_{H} 1.29) to C-25 (δ_{C} 42.4) and C-26 (δ_{C} 173.0). Moreover, the HMBC correlations from δ_{H} 1.65 (H-19), 6.24 (H-2), and 7.19 (H-3) to δ_{C} 202.9 (C-1) demonstrated the presence of a conjugated 2-en-1-one group, and the ^1H - ^1H COSY correlations of δ_{H} 7.19 (H-3)/ δ_{H} 4.01 (H-4) revealed that there existed an hydroxyl group at C-4. An oxymethine in **1** instead of a methene in physalin J suggests that one hydroxyl group could be located at C-7, which was confirmed by the ^1H - ^1H COSY correlations of δ_{H} 3.54 (H-6)/ δ_{H} 5.70 (H-7) and δ_{H} 5.70 (H-7)/ δ_{H} 2.93 (H-8). The relative configurations of H-8 and Me-28 in the physalin skeleton were established to be β -oriented by X-ray.^{20,21} Thus, the NOE correlations (Figure 1) of H-4/H-6, H-6/H-7, H-7/H-8 β , and H-27/Me-28 established the relative configuration of OH-4 and OH-7 to be α -oriented and Me-27 to be β -oriented. Hence, the structure of compound **1** was identified to be physalin III (**1**).

Figure 1. Key HMBC (H→C) and ROESY(H ↔ H) correlations of compound **1**

Compound **2** was obtained as a colorless needle crystal. The molecular formula of **2** was determined to be C₂₈H₃₂O₁₂ on the basis of HREIMS (*m/z* 560.1854, [M]⁺; calcd 560.1894) and NMR data (Table 1). The NMR data of **2** were similar to those of physalin Z²², and the differences between them were two of the double-bond carbon signals in physalin Z were replaced by

two oxygenated methine signals in **2** located at C-6 and C-7, which were deduced by ^1H - ^1H COSY correlations of H-8 (δ_{H} 3.39)/H-7 (δ_{H} 5.23) and H-7 (δ_{H} 5.23)/H-6 (δ_{H} 4.79) and by HMBC correlations from H-6 to C-10, C-4, C-8, C-7, and C-5, from H-7 to C-6, C-5, C-8, and C-14. The ROESY correlations (Figure 2) of H-6/H-7 and H-7/H-8 β revealed that OH-C(6) and OH-C(7) were α -oriented. So the structure of compound **2** was deduced and named as physalin IV (**2**).

Figure 2. Key HMBC (H→C) and ROESY(H ↔ H) correlations of compound **2**

Compound **3** was obtained as an amorphous yellow powder. The molecular formula of **3** was determined as C₂₉H₃₂O₁₀ using HREIMS and NMR data (Table 1). These NMR data of **3** were similar to those of physalin X²³, except the presence of an additional methoxyl group. Comparing with physalin X, the chemical shift of C-3 (δ_{C} 76.7) at down field and the HMBC correlation (Figure 3) of OMe (δ_{H} 3.27) with C-3 in **3** indicated a methoxyl group at C-3. The relative configuration of Me-19 and H-16 in physalin skeleton were established to be β -oriented by X-ray.^{20,21} So the relative configuration of 3-OMe could be determined as α -oriented by the NOE correlations of Me-19/H-3 and H-16/H-3. Hence, the structure of compound **3** was identified to be 3-*O*-methylphysalin X (**3**).

The structures of five known steroidal compounds, physalin Z (**4**)²², physalin C (**5**)²⁴, physalin I (**6**)²⁵, physalin P (**7**)²⁶, 25,27-dihydro-4,7-dedehydro-7-deoxyneophysalin A (**8**)²⁷, were elucidated in comparison to their spectroscopic data with those reported in the literature.

Figure 3. Key HMBC (H→C) and ROESY(H ↔ H) correlations of compound **3**

Experimental Section

General Experimental Procedures. Column chromatography (CC): silica gel (100–200 mesh, 200–300 mesh; Qingdao Marine Chemical Co., Ltd.), Sephadex LH-20 (Pharmacia). Thin-layer chromatography (TLC): silica gel 60F₂₅₄ (Qingdao Marine Chemical Co., Ltd.). Optical rotations: Jasco P-1020 polarimeter. UV Spectra: Shimadzu UV-2401 PC spectrophotometer. IR Spectra: Bruker Tensor-27 infrared spectrophotometer, with KBr pellets in cm⁻¹. NMR Spectra: Bruker Bruker Avance III-600MHz spectrometer; chemical shifts δ were recorded as ppm relative to TMS, coupling constants J in Hz. ESIMS: API QSTAR Pulsar spectrometer; HREIMS: Waters Autospec Premier P776.

Plant Material. The calyces of *Phsalis alkekengi* var. *franchetii* were purchased in Kunming CTM market, Kunming city, Yunnan province at December 2011 and were identified by Prof. Xi-Wen Li who was botanic taxonomist in Kunming Institute of Botany.

Extraction and Isolation. Dried calyces (15 kg) of *P. alkekengi* var. *franchetii* were extracted with 80% EtOH under reflux. The extract was suspended in water (5 L) and then partitioned successively with PE (petroleum ether) (8 L × 4) and EtOAc (8 L × 5). The EtOAc-soluble layer (evaporated under vacuum to get 250 g) eluted successively with CHCl₃, CHCl₃-MeOH (100:1), CHCl₃-MeOH (20:1), and CHCl₃-MeOH (5:1). Then, the CHCl₃-MeOH (100:1) portion (80 g) was chromatographed on a silica gel column using a gradient of CHCl₃-CH₃OH (100:0 to 0:100), which yielded five fractions (1–5). Fraction 2 was successively subjected to RP-18, Sephadex LH-20 and silica gel to derive subfractions I–IV. Subfraction II was further separated over silica gel with CHCl₃-CH₃OH (120:1) to yield compound **3** (5 mg), **6** (4 mg), **7** (10 mg) and **8** (3 mg). Subfraction III was subjected to semi-preparative HPLC (CH₃CN-H₂O, 50:50) to produce **4** (1 mg) and **5** (1 mg). Fraction 3 was successively subjected to RP-18, Sephadex LH-20 and separated over silica gel with CHCl₃-CH₃OH (80:1) to yield **1** (2 mg) and **2** (6 mg).

Physalin III (1): white powder; $[\alpha]_D^{21}$ – 29.33 (*c* 0.2, CHCl₃); UV (CHCl₃): $\lambda_{\max}$ (log ϵ): 240 (3.36) nm; IR (KBr):

$\nu_{\max}$ 3430, 2924, 1787, 1758, 1728, 1462, 1376, 1218, 1073, 1041 cm⁻¹; ¹H and ¹³C NMR: see Table 1. Negative ESIMS: 559 ([M – H]⁻). HREIMS: 560.1885 [M]⁻ (calcd. for C₂₈H₃₂O₁₂, 560.1894).

Physalin IV (2): colorless needle crystal (CHCl₃); $[\alpha]_D^{16}$ – 114.40 (*c* 0.1, MeOH); UV (MeOH): $\lambda_{\max}$ (log ϵ): 201 (4.23) nm; IR (KBr): $\nu_{\max}$ 3431, 1781, 1729, 1632, 1383, 1374, 1167, 1134, 1065 cm⁻¹; ¹H and ¹³C NMR: see Table 1. Positive ESIMS: 583 ([M + Na]⁺). HREIMS: 560.1854 [M]⁺ (calcd. for C₂₈H₃₂O₁₂, 560.1894).

3-O-Methylphysalin X (3): yellow powder; $[\alpha]_D^{18}$ – 16.00 (*c* 0.1, pyridine); UV (MeOH): $\lambda_{\max}$ (log ϵ): 225 (3.77), 201 (3.80) nm; IR (KBr): $\nu_{\max}$ 3432, 2923, 1786, 1629 cm⁻¹; ¹H and ¹³C NMR: see Table 1. Positive ESIMS: 563 ([M + Na]⁺). HREIMS: 540.1933 [M]⁺ (calcd. for C₂₉H₃₂O₁₀, 540.1995).

Electronic Supplementary Material

Supplementary material is available in the online version of this article at <http://dx.doi.org/10.1007/s13659-013-0021-z> and is accessible for authorized users.

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