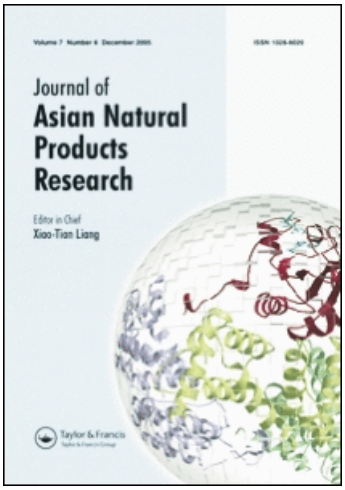

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

A pair of 3-epimeric physalins from *Physalis alkekengi* L. var. *franchetii*

Li Qiu[a]; Zhi-Hu Jiang[a]; Hong-Xia Liu[a]; Li-Xia Chen[a]; Xin-Sheng Yao[a]; Feng Qiu[a]
[a] Department of Natural Products Chemistry, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China

To cite this Article Qiu, Li , Jiang, Zhi-Hu , Liu, Hong-Xia , Chen, Li-Xia , Yao, Xin-Sheng and Qiu, Feng(2008) 'A pair of 3-epimeric physalins from *Physalis alkekengi* L. var. *franchetii*', Journal of Asian Natural Products Research, 10: 9, 881 — 885
To link to this Article: DOI: 10.1080/10286020802144735
URL: http://dx.doi.org/10.1080/10286020802144735

Journal of Asian Natural Products Research,
Vol. 10, No. 9, September 2008, 881–885

Taylor & Francis
Taylor & Francis Group

A pair of 3-epimeric physalins from *Physalis alkekengi* L. var. *franchetii*

Li Qiu, Zhi-Hu Jiang, Hong-Xia Liu, Li-Xia Chen, Xin-Sheng Yao and Feng Qiu*

Department of Natural Products Chemistry, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China

(*Received 20 November 2007; final version received 3 April 2008*)

A pair of new 3-epimeric steroids possessing a rare 13,14-seco-16,24-cycloergostane skeleton, 3α-methoxy-2,3-dihydro-4,7-didehydrophysalin B (**1**), and 3β-methoxy-2,3-dihydro-4,7-didehydrophysalin B (**2**) were isolated from the 60% EtOH extract of the stems and leaves of *Physalis alkekengi* L. var. *franchetii*. Their structures were characterized on the basis of spectral evidence.

Keywords: Solanaceae; *Physalis alkekengi* L. var. *franchetii*; 3α-methoxy-2,3-dihydro-4,7-didehydro-physalin B; 3β-methoxy-2,3-dihydro-4,7-didehydrophysalin B

1. Introduction

The calyces of *Physalis alkekengi* L. var. *franchetii* (Chinese name: Jindenglong) are used as a traditional Chinese herbal medicine for the treatment of sore throat, cough, eczema, hepatitis, urinary problems, and tumors [1]. Physalins, a class of steroidal constituents from *P. alkekengi* L. var. *franchetii*, which possess a rare 13,14-seco-16,24-cycloergostane skeleton, are known to be one of the major bioactive components. Pharmacological studies demonstrated that physalins have antimycobacterial [2], immunomodulatory [3], anti-tumor [4], and anti-inflammatory [5] activities. The unusual steroids and variety of biological activities of *P. alkekengi* L. var. *franchetii* prompted us to extend our studies to this plant. In our previous study, we reported four known steroidal constituents, physalins D, L, O, and 4,7-didehydroneophysalin B from the calyces [6], and further investigation on the stems and leaves of *P. alkekengi* L. var. *franchetii* led to the isolation of a pair of new 3-epimeric physalins. This paper describes the isolation and structural elucidation of the two new compounds.

2. Results and discussion

Compound **1** was a yellowish powder and its molecular formula, $C_{29}H_{32}O_{10}$, was determined based on HRESIMS at *m/z* 563.1888 $[M + Na]^+$ in combination with the ^1H and ^{13}C NMR spectral data. The ^1H NMR spectrum showed signals for three methyls at δ 0.99 (3H, s, H-19), 1.16 (3H, s, H-28), and 1.77 (3H, s, H-21), one oxygenated methylene at δ 4.35 (1H, dd, $J = 13.6$, 4.6 Hz, H-27), and 3.66 (1H, d, $J = 13.6$ Hz, H-27). The ^{13}C NMR spectrum displayed carbon signals for two ketonic carbonyls at δ 212.2 (C-1) and 208.6 (C-15), two lactonic carbonyls at δ 171.6 (C-18) and 167.3 (C-26), three oxygenated quaternary carbons at δ 78.8 (C-13), 80.4 (C-20), and 80.9 (C-17), one oxygenated methine carbon at δ 76.5 (C-22), and one oxygenated methylene

*Corresponding author. Email: fengqiu2000@tom.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802144735
http://www.informaworld.com

carbon at δ 61.4 (C-27). The NMR spectral data of **1** were closely comparable to those of physalin B,[7] suggesting that **1** was also a physalin compound, and the difference between them was the substituent pattern in A/B rings. The NMR spectral data of **1** displayed a conjugated diene system composed of a disubstituted double bond (δ 6.24, 6.00 and 128.8, 125.9) and a trisubstituted double bond (δ 5.74 and 142.2, 125.4) in addition to a methoxyl group (δ 3.27 and 55.5). The HMBC correlations from H-4 at δ 5.74 to C-2, C-10, and C-6, from H-7 at δ 6.00 to C-5, C-9, and from H-6 at δ 6.24 to C-4, C-10, and C-8 corroborated the conjugated diene system was located at C-4 and C-5, C-6, and C-7. The HMBC correlations from CH_3O-3 (δ 3.27) to C-3, from H-2β(δ 2.93), H-2α (δ 2.45) to C-3, C-1, and from H-3 (δ 4.23) to CH_3O-3, C-1 indicated that the methoxyl group was located at C-3. The NOESY spectrum of **1** showed correlations of H-19 and H-2β, H-3 and H-2β, CH_3O-3 and H-2α, indicating the α-configuration of 3-methoxyl group. Thus, the structure of **1** was characterized to be 3α-methoxy-2,3-dihydro-4,7-didehydrophysalin B (Figure 1).

Compound **2** was a yellowish powder with a molecular formula of $C_{29}H_{32}O_{10}$ established by HRESIMS at *m/z* 563.1888 $[M + Na]^+$ in combination with the ^1H and ^{13}C NMR spectral data. Comparison of the ^1H and ^{13}C NMR spectral data of **2** with those of **1** suggested that **2** was the 3-epimer of **1**. This conclusion was further supported by the HMBC correlations from CH_3O-3 to C-3, and H-3 to CH_3O-3, C-1, C-4, and C-5. Severely, overlapping of the signals of H-2α and H-2β in the ^1H NMR spectrum (in DMSO-d_6) of **2** disturbed the determination of the configuration of the CH_3O-3, thus, the ^1H, ^{13}C, HSQC, HMBC, and NOESY spectra of **2** were remeasured in $CDCl_3$. The NOESY spectrum (in $CDCl_3$) of **2** exhibited NOE correlations of H-19 (δ 1.38) with H-2β (δ 3.06), and H-3 (δ 4.23) with H-2α (δ 2.88), supporting the β-configuration of the 3-methoxyl group. Therefore, the structure of **2** was established to be 3β-methoxy-2,3-dihydro-4,7-didehydrophysalin B (Figure 2).

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Yanaco Mp-S3 micromelting point apparatus and are uncorrected. UV spectra were recorded with a SHIMADZU UV 2201 spectrophotometer, and optical rotations were measured with a Perkin-Elmer 241 polarimeter. NMR spectra were performed on Bruker ARX 600 spectrometer, using TMS as an internal standard. ESI-MS data were measured on an Agilent 1100-LC/MSDTrapSL mass spectrometer and HR-ESI-MS data were measured on an Agilent 6210 TOF mass spectrometer. Silica gel GF254 prepared for TLC and silica gel (200–300 mesh) for column chromatography (CC) were obtained from Qingdao Marine Chemical Company, Qingdao, China. Sepha-

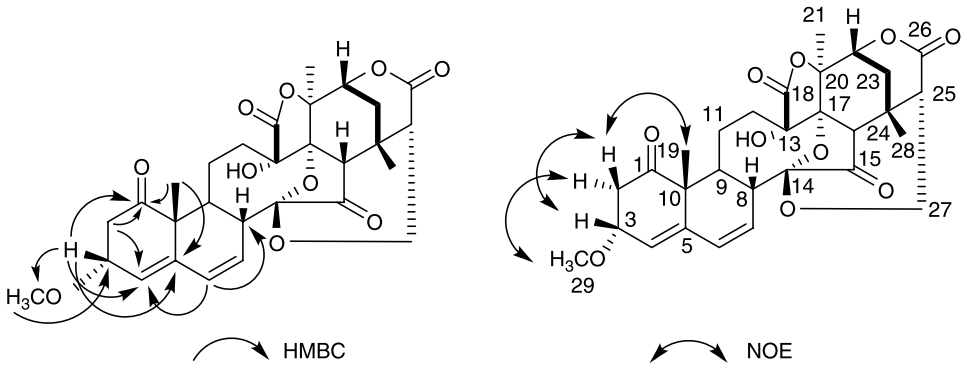

Figure 1. Selected HMBC and NOESY correlations of compound **1**.

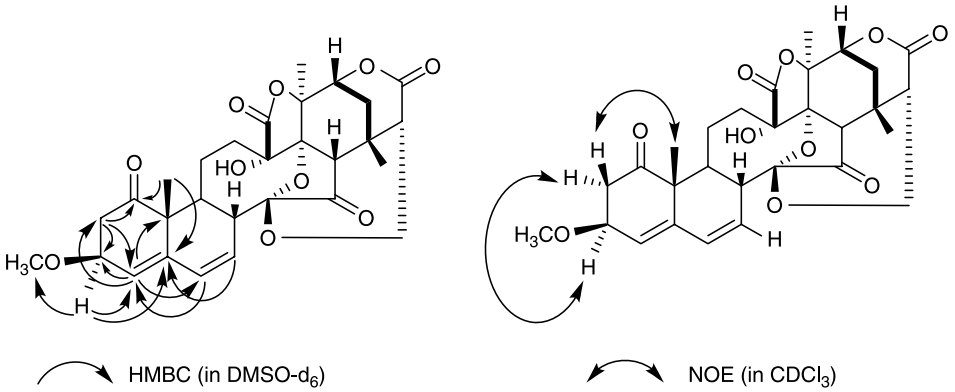

Figure 2. Selected HMBC and NOESY correlations of compound **2**.

dex LH-20 was a product of Pharmacia Co., Sweden. Rp-18 (40–75 mm) silica gel was purchased from Merck Chemical Ltd., Darmstadt, Germany. Preparative HPLC (PHPLC) was carried on a Waters 600 apparatus with a preparative reversed phase C18 column (Inertsil Prep-ODS 20 × 250 mm, 10 μm, GL. Sciences Inc., Torrance, USA).

3.2 Plant material

The dried stems and leaves of *P. alkekengi* L. var. *franchetii* were collected in September 2004, in the locality of Yilan, Heilongjiang Province, China and were authenticated by Prof. Qishi Sun, Department of Pharmaceutical Botany, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University. A voucher specimen (Collection No. 20041018) has been deposited in the herbarium of the Laboratory of Natural Products Chemistry, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

The dried stems and leaves (7 kg) of *P. alkekengi* L. var. *franchetii* were extracted with 60% EtOH (20 L × 2) under reflux and about 1000 g extract was obtained, which was suspended in water and then partitioned with cyclohexane, EtOAc, and *n*-BuOH successively. The EtOAc extract was evaporated under vacuum to give a gummy solid (180 g). EtOAc extract (180 g) was chromatographed on silica gel column using gradient solvents of $CHCl_3$-MeOH as eluents to yield fractions 1–9. Fraction 3 was subjected to silica gel column, eluting with gradient solvents of cyclohexane–EtOAc system to give fractions 31–39. Fraction 36 was chromatographed on Sephadex LH-20 [$CHCl_3$–MeOH (1:1)] to yield three subfractions (fractions 361–363). Fraction 363 was submitted to preparative HPLC [MeCN/H_2O (45:55), flow rate 8.0 ml/min] to yield fraction 3631 and fraction 3632, both of which were further purified by preparative TLC [cyclohexane-EtOAC (1:2)] to obtain **1** (27 mg, $R_f = 0.60$) and **2** (15 mg, $R_f = 0.63$), respectively.

3.3.1 3α-Methoxy-2,3-dihydro-4,7-didehydrophysalin B (*1*)

Yellowish powder, mp 265–266°C; $[\alpha]^{20}_D$ − 55.0 ($c = 0.14$, MeCN); UV (MeCN) λ_{max} nm (log ε): 232 (4.20); ^{1}H and ^{13}C NMR (DMSO-d_6) spectral data (see Table 1); ESIMS *m/z*: 575 $[M + Cl]^-$, 563 $[M + Na]^+$; HRESIMS *m/z*: 563.1888 $[M + Na]^+$ (calcd for $C_{29}H_{32}O_{10}Na$, 563.1893).

3.3.2. 3β-Methoxy-2,3-dihydro-4,7-didehydrophysalin B (*2*)

Yellowish powder, mp 234–235°C; $[\alpha]^{20}_D$ − 106.0 ($c = 0.10$, MeCN); UV (MeCN) λ_{max} nm (log ε): 229 (4.20); ^{1}H and ^{13}C NMR (DMSO-d_6) spectral data (see Table

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (δ in ppm, J in Hz, 600 MHz for ^{1}H and 150 MHz for ^{13}C).

	1 (in DMSO-d_6)		**2** (in DMSO-d_6)		**2** (in $CDCl_3$)	
No.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	212.2		211.0		213.3	
2	43.7	(α) 2.45 dd, 12.7, 7.5 (β) 2.93 dd, 12.7, 5.8	43.4	(α) 2.66 m (β) 2.66 m	41.8	(α)2.88 dd, 12.2, 8.0 (β)3.06 dd, 12.2, 7.1
3	75.8	4.23 br t, 6.5	74.2	4.15 dd, 9.5, 4.7	77.8	4.23 dt, 3.4, 7.4
4	125.4	5.74 br s	122.8	5.87 d, 5.3	124.0	5.64 d, 3.2
5	142.2		145.1		142.1	
6	128.8	6.24 dd, 10.3, 2.8	129.0	6.25 dd, 10.2, 2.7	128.0	6.14 dd, 10.3, 2.3
7	125.9	6.00 d, 10.3	126.4	6.02 d, 10.2	126.7	6.19 br d, 11.0
8	44.9	2.65 m	45.0	2.65 m	44.2	2.75 br d, 10.5
9	33.9	3.10 m	33.6	3.05	32.2	2.85 m
10	50.3		50.3		51.4	
11	22.1	1.78 m 0.90 m	21.6	1.93 m 0.89 m	25.3	1.59 m 1.24 m
12	24.5	1.96 m 1.38 m	24.5	1.90 m 1.37 m	25.9	2.32 m 2.32 m
13	78.8		78.7		80.1	
14	105.7		105.7		106.4	
15	208.6		208.6		207.8	
16	53.6	2.86 s	53.4	2.87 s	55.6	2.18 s
17	80.9		80.9		81.0	
18	171.6		171.6		171.9	
19	16.4	0.99 s	17.2	0.99 s	20.6	1.38 s
20	80.4		80.4		80.6	
21	21.7	1.77 s	21.7	1.75 s	21.4	1.95 s
22	76.5	4.58 m	76.4	4.57 m	77.0	4.54 m
23	31.2	2.12 dd, 11.0, 3.5 1.93 m	31.2	2.12 dd, 14.5, 3.4 1.93 m	32.9	2.04 m 2.04 m
24	30.7		30.7		31.2	
25	49.3	2.96 d, 4.1	49.3	2.96 d, 4.3	50.9	2.48 d, 4.3
26	167.3		167.2		166.4	
27	61.4	4.35 dd, 13.6, 4.6 3.66 d, 13.6	61.4	4.34 dd, 13.6, 4.5 3.67 d, 13.6	61.2	4.62 dd, 13.4, 4.6 3.83 d, 13.4
28	24.4	1.16 s	24.4	1.16 s	26.5	1.27 s
29	55.5	3.27 s	55.5	3.21 s	56.2	3.40 s
(3-OCH_3)	6.50 s					
13-OH				6.56 s		

1); ESIMS *m/z*: 539 $[M - H]^-$, 541 $[M + H]^+$; HRESIMS *m/z*: 563.1888 $[M + Na]^+$ (calcd for $C_{29}H_{32}O_{10}Na$, 563.1893).

Acknowledgements

The authors are grateful to Ms. Wen Li and Mr. Yi Sha of the Department of Instrumental Analysis, Shenyang Pharmaceutical University for measuring the NMR and MS spectra. The authors also thank Dr. Deqiang Dou, College of Pharmacy, Liaoning University of Traditional Chinese Medicine for his kind help in measuring HRESIMS spectra.

References

[1] Chinese Pharmacopoeia Committee, in *Pharmacopoeia of The People's Republic of China*, (Chemical Industry Press, Beijing, 2005), Vol. **1**, p. 250.

[2] A.H. Januário, E.R. Filho, R.C.L.R. Pietro, S. Kashima, D.N. Sato, and S.C. França, *Phytother. Res.* **16**, 445 (2002).

[3] M.B.P. Soares, M.C. Bellintani, I.M. Ribeiro, T.C.B. Tomassini, and R.R.D. Santos, *Eur. J. Pharmacol.* **459**, 107 (2003).

[4] H.I.F. Magalhães, M.L. Veras, M.R. Torres, A.P.N.N. Alves, O.D.L. Pessoa, L.V. Costa-

Lotufo, M.O.D. Moraes, and C. Pessoa, *J. Pharm. Pharmacol.* **58**, 235 (2006).

[5] A.T. Vieira, V. Pinho, L.B. Lepsch, C. Scavone, I.M. Ribeiro, T. Tomassini, R. Ribeiro-dos-Santos, M.B.P. Soares, M.M. Teixeira, and D.G. Souza, *Br. J., Pharmacol.* **146**, 244 (2005).

[6] Q. Zhao, L. Qiu, G.M. Bu, G.X. Qu, and F. Qiu, *J. Shenyang Pharm. Univ.* **23**, 151 (2006).

[7] M. Kawai, T. Ogura, B. Makino, A. Matsumoto, H. Yamamua, Y. Butsugan, and M. Hayashi, *Phytochemistry* **31**, 4299 (1992).