Two Novel Neophysalins from *Physalis alkekengi* L. var. *franchetii*

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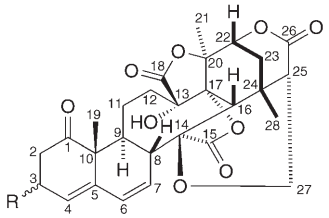
Two novel neophysalins, named physalin W (**1**) and physalin X (**2**), were isolated from the EtOH extract of the aerial parts of *Physalis alkekengi* L. var. *franchetii* (Solanaceae). Their structures were determined mainly by spectroscopic techniques including 2D-NMR (HMBC, HSQC, ^{1}H,^{1}H-COSY, NOESY) and MS experiments.

Introduction. – Physalins are characterized by the presence of the 16,24-cyclo-13,14-seco-ergostane skeleton and have been commonly isolated from plants of the genus *Physalis* [1–6]. Several *Physalis* species have been considered of a great medicinal value, since compounds isolated from them display a wide spectrum of biological activities such as antimicrobial, antitumor, anti-inflammatory, immunomodulatory, cytotoxic, immunosuppressive, trypanocidal, and molluscicidal effects [7–14].

For centuries, *Physalis alkekengi* L. var. *franchetii* (Solanaceae) has been well-known for its use in traditional Chinese medicine. In the course of our study on the constituents of *P. alkekengi* L. var. *franchetii*, two novel neophysalins, **1** and **2**, were isolated. They possess the neophysalin skeleton [15] which, up to now, has been present in but one natural product, namely in physalin P [16]. We describe the structure determination of the two novel compounds **1** and **2** possessing this skeleton.



Results and Discussion. – Physalin W (**1**) was obtained as an amorphous yellow powder. The ESI-MS of **1** showed a pseudomolecular ion $[M+NH_4]^+$ at *m*/*z* 544. Taking into account the 28 C-atoms displayed in its ^{13}C-NMR spectrum, the molecular formula was established as $C_{28}H_{30}O_{10}$. All ^{1}H- and ^{13}C-NMR signal assignments (*Table*) were confirmed by 2D-NMR techniques, and the structure of **1** was established as (3*β*,14*α*,16*β*,22*α*,25*S*)-14,27-epoxy-3,13,17,20,22-pentahydroxy-1-oxo-14,16 : 16,24-cyclo-

13,14-secoergosta-4,6-diene-15,18,26-trioic acid, γ-lactone γ-lactone δ-lactone, and named physalin W.

Table. *^{1}H-and ^{13}C-NMR Data of* **1** *and* **2**. At 500 (125) MHz in (D_6)DMSO; δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		211.3		210.0
CH_2(2)	2.79 (*dd*, J = 12.3, 5.8, H_α), 2.43 (*dd*, J = 12.3, 7.5, H_β)	46.7	2.62 (*dd*, J = 5.5, 1.7, H_α), 2.61 (*dd*, J = 5.5, 2.1, H_β)	45.6
H–C(3)	4.46 (br. *s*)	66.4	4.37 (br. *s*)	66.3
OH–C(3)	5.23 (*d*, J = 4.8)		5.17 (*d*, J = 1.8)	
H–C(4)	5.55 (*d*, J = 2.3)	127.6	5.60 (*d*, J = 4.5)	124.9
C(5)		139.5		140.9
H–C(6)	6.15 (*dd*, J = 10.4, 2.1)	126.6	6.15 (*dd*, J = 10.3, 2.0)	126.6
H–C(7)	6.06 (*dd*, J = 10.4, 3.0)	127.5	6.06 (*dd*, J = 10.3, 3.0)	127.5
H–C(8)	2.98–2.99 (*m*)	47.9	3.01–3.03 (*m*)	47.4
H–C(9)	2.00–2.01 (*m*)	36.5	1.98–2.00 (*m*)	35.8
C(10)		50.6		50.7
CH_2(11)	1.84–1.87, 1.69–1.73 (2*m*)	21.4	1.88–1.92, 1.48–1.55 (2*m*)	21.6
CH_2(12)	2.17–2.23, 2.03–2.08 (2*m*)	28.5	2.18–2.21, 2.05–2.08 (2*m*)	28.1
C(13)		78.3		78.3
OH–C(13)	6.58 (*s*)		6.54 (*s*)	
C(14)		81.2		81.3
C(15)		170.8		171.0
H–C(16)	2.96 (*s*)	47.3	2.96 (*s*)	47.3
C(17)		82.6		82.7
C(18)		172.8		172.7
Me(19)	1.07 (*s*)	17.5	1.18 (*s*)	19.0
C(20)		82.3		82.2
Me(21)	1.65 (*s*)	21.3	1.65 (*s*)	21.3
H–C(22)	4.59 (*dd*, J = 4.2, 1.6)	76.0	4.58 (*dd*, J = 4.2, 1.6)	76.0
CH_2(23)	2.00–2.06 (*m*), 1.84 (*dd*, J = 14.6, 1.6)	29.7	2.01–2.05 (*m*), 1.83 (*dd*, J = 14.8, 1.6)	29.7
C(24)		28.3		28.5
H–C(25)	2.99–3.01 (*m*)	39.2	2.98–2.99 (*m*)	39.0
C(26)		170.4		170.4
CH_2(27)	4.31 (*t*, J = 12.4), 3.96 (*dd*, J = 12.4, 3.9)	60.5	4.30 (*t*, J = 12.5), 3.92 (*dd*, J = 12.5, 3.8)	60.6
Me(28)	1.36 (*s*)	29.5	1.36 (*s*)	29.5

The 500-MHz ^{1}H-NMR spectrum of **1** in (D_6)DMSO showed the presence of two OH groups (δ 6.58 and 5.23), three tertiary Me groups (δ 1.07, 1.36, and 1.65) and three olefinic protons (δ 5.55 (*d*, J = 2.3 Hz), 6.06 (*dd*, J = 10.4, 3.0 Hz), and 6.15 (*dd*, J = 10.4, 2.1 Hz)). In the ^{13}C-NMR spectrum of **1**, the signals of a ketone group (δ 211.3) and three lactone groups (δ 170.4, 170.8, and 172.8) were present but no signals of a ketal group characteristic for physalin derivates. This implied that the structure of **1** had a structure different from that of common physalin derivates, namely a neophysalin skeleton, which is considered to be derived from the physalin structure by a benzilic acid rearrangement. The ^{13}C-NMR spectrum of **1** showed the presence of four olefinic C-atoms (δ 126.6, 127.5, 127.6, and 139.5), and seven C-atoms attached to the O-functions (δ 60.5, 66.4, 76.0, 78.3, 81.2, 82.3, and 82.6). The HMBC and HSQC

spectra of **1** established that the four olefinic C-atoms belong to a conjugated diene system within the *A*/*B* ring system. The ^{1}H- and ^{13}C-NMR spectra of **1** showed the presence of a C(27)–O–C(14) ether bridge (δ 60.5 and 81.2; δ 4.31 (*t*, J = 12.4 Hz) and 3.96 (*dd*, J = 12.4, 3.9 Hz)). These facts indicated that the structure of **1** was of the neophysalin-B type. The HMBC correlations of the methylene protons (δ 2.79 and 2.43) with C(1) (δ 211.3), with an OH-substituted C-atom (δ 66.4), and with an olefinic C-atom (δ 127.6) inferred that the C(2)–C(3) bond was saturated and that the OH group was present at C(3) (*Fig. 1,a*). Concerning the relative configuration of **1**, the NOESY cross-peaks (*Fig. 1,b*) between H–C(3) and H_α–C(2) and the coupling constants of H–C(3) and H–C(2) ($J(2\alpha,3) = 5.8$ and $J(2\beta,3) =$ 7.5 Hz) indicated that H_α–C(3) is axial.

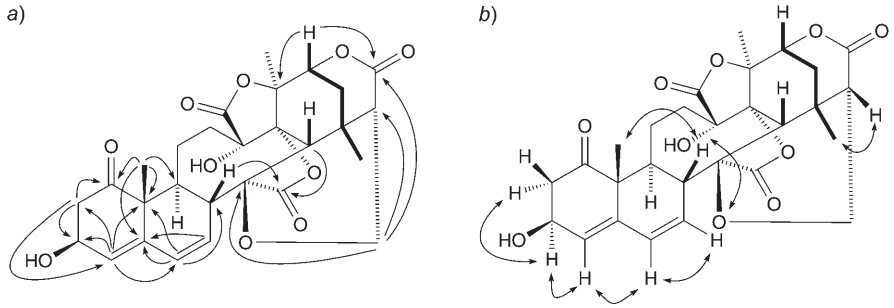


Fig. 1. a) *Key HMBC and* b) *key NOESY interactions in* **1**

Physalin X (**2**) was obtained as an amorphous yellow powder. The ESI-MS of **2** also showed the pseudomolecular ion $[M + NH_4]^+$ at *m*/*z* 544, so the molecular formula was established to be $C_{28}H_{30}O_{10}$. The ^{13}C-NMR spectrum of **2** was very similar to that of **1**, suggesting that **2** had the same neophysalin skeleton as **1**. The assignment of all ^{1}H- and ^{13}C-NMR signals (*Table*) were confirmed by 2D-NMR techniques, and the structure of **2** was established as (3α,14α, 16β,22α,25*S*)-14,27-epoxy-3,13,17,20,22-pentahydroxy-1-oxo-14,16 : 16,24-cyclo-13,14-secoergosta-4,6-diene-15,18,26-trioic acid, γ-lactone γ-lactone δ-lactone, and named physalin X (*Fig. 2*).

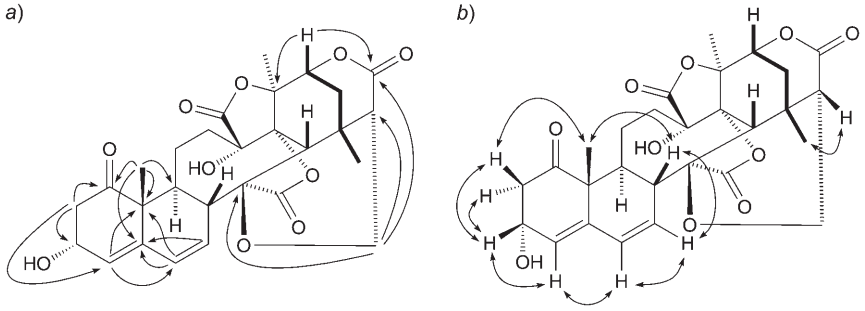


Fig. 2. a) *Key HMBC and* b) *key NOESY interactions in* **2**

The ^{13}C-NMR chemical shift difference between the corresponding C-atoms of **1** and **2** were less than 0.4 ppm for C(11) to C(28), except for C(19). Therefore, **2** was assumed to differ from **1** in the *AB* ring moiety. Significant differences were also observed for the proton resonances assignable to the *AB* ring moiety. For example, Me(19) of **2** (δ 1.18) resonated at a lower field than that of **1** (δ 1.07). This might be due to the different configuration of OH–C(3). There were NOESY cross-peaks not only

between H–C(3) and H_α–C(2) but also between H–C(3) and H_β–C(2) (*Fig. 2,b*). And the coupling constants of H–C(2) and H–C(3) were much smaller ($J(2\alpha,3) = 1.7$ and $J(2\beta,3) = 2.1$ Hz) indicating an equatorial H–C(3), *i.e.*, an α-axial orientation of OH–C(3). Accordingly, **2** was deduced to be an epimer of **1** with respect to the relative configuration of OH–C(3).

Experimental Part

General. All solvents used were of anal. grade (*Tianjing Chemical Plant*). Column chromatography (CC): silica gel *H* (100–200 mesh, 200–300 mesh; *Qingdao Marine Chemical Ltd.*), *Sephadex LH-20* (25–100 μm; *Pharmacia*), and *RP-18* (20–45 μm; *Fuji Silysia Chemical Ltd.*). Thin-layer chromatography (TLC): silica gel GF_{254} (*Yantai Huiyou*). ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Spectra: *Bruker AMX-500* spectrometer; δ in ppm, *J* in Hz. ESI-MS: *Agilent 1100-MSD*-trap spectrometer; in *m*/*z*.

Plant Material. The aerial parts of *P. alkekengi* L. var. *franchetii* were collected in JiLin Province, China, and identified by *Xue-Hua Song*, the curator of TCM Specimen Hall of China Pharmaceutical University (P. R.China).

Extraction and Isolation. The aerial parts (9.5 kg) of *P. alkekengi* L. var. *franchetii* were extracted with hot EtOH, which afforded a dark residue (255.4 g) after concentration. The residue was partitioned between $CHCl_3$ and H_2O. The org. extract (102.6 g after evaporation) was subjected to CC (silica gel *H* (100–200 mesh), $CHCl_3$/MeOH 50:1, 20:1, 10:1, 5:1, 2:1): *Fractions A–E. Fr. B* (23.9 g) was subjected to CC (silica gel *H* (200–300 mesh), petroleum ether/AcOEt 9:1, 5:1, 2:1, 1:1): *Fr. B.1–B.4*. *Fr*actions *B.4* was resubjected to CC (*RP-18*, MeOH/H_2O 1:1): **1** (2.4 mg) and **2** (6.5 mg).

Physalin W (=*(3β,14α,16β,22α,25*S*)-14,27-Epoxy-3,13,17,20,22-pentahydroxy-1-oxo-14,16:16,24-cyclo-13,14-secoergosta-4,6-diene-15,18,26-trioic acid*, *γ-Lactone γ-Lactone δ-Lactone*; **1**): Amorphous, yellow powder. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 544 ($[M+NH_4]^+$, $C_{28}H_{34}NO_{10}^+$).

Physalin X (=*(3α,14α,16β,22α,25*S*)-14,27-Epoxy-3,13,17,20,22-pentahydroxy-1-oxo-14,16:16,24-cyclo-13,14-secoergosta-4,6-diene-15,18,26-trioic acid*, *γ-Lactone γ-Lactone δ-Lactone*; **2**): Amorphous, yellow powder. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 544 ($[M+NH_4]^+$, $C_{28}H_{34}NO_{10}^+$).

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