Two New Withanolides from *Physalis peruviana*

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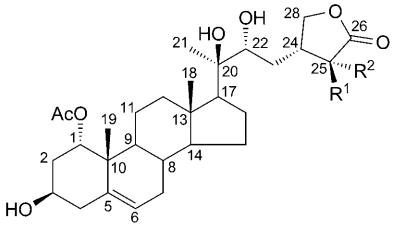
Two new perulactone-type withanolides, named perulactone C (**1**) and perulactone D (**2**), together with four known compounds, perulactone (**3**), perulactone B (**4**), blumenol A, and (+)-(*S*)-dehydrovomifoliol, were isolated from the aerial parts of *Physalis peruviana*. The structures of the new compounds were elucidated on the basis of 1D- and 2D-NMR experiments, including HMBC, HSQC, ^{1}H,^{1}H-COSY, and ROESY, as well as HR-MS.

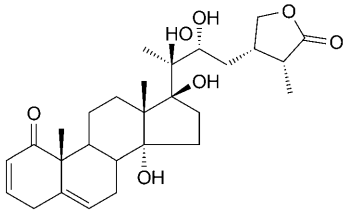
Introduction. – The genus *Physalis* (family Solanaceae) includes about 120 species, and most of them growing in South and North America. A small number of species has distributed in Europe and in the countries of southeastern and central Asia. Five species of *Physalis* are found in China [1]. *Physalis peruviana* is a common plant in China, called cape gooseberry (Chinese name: deng-long-guo) as an edible fruit. It is also a medicinal plant widely used in folk medicine for treating diseases such as malaria, asthma, hepatitis, dermatitis, diuretic diseases, and rheumatism [2][3].

The withanolides are steroidal lactones with an ergostane skeleton containing 28 C-atoms. Most withanolide compounds are produced by Solanaceae plants, in particular by the genera *Physalis*, *Withania*, *Datura*, *Nicandra*, *Dunalia*, *Lycium*, *Tubocapsicum*, and *Jaborosa* [4]. Such compounds often have antimicrobial, antitumor, antiinflammatory, hepatoprotective, immunomodulatory, and insect-repellent properties [4]. Due to our interest in the biological properties of these compounds, we investigated withanolides from *Physalis peruviana*. In this paper, we report the isolation and structure elucidation of two new perulactones, perulactone C (**1**) and perulactone D (**2**) from the aerial parts of *Physalis peruviana*, along with two known withanolides, perulactone (**3**) and perulactone B (**4**), and two known *nor*-isoprenoids, blumenol A, and (+)-(*S*)-dehydrovomifoliol. In previous investigations, only two known perulactone-type withanolides have been isolated from *Physalis peruviana* [5][6].

Results and Discussion. – Compound **1**, named perulactone C, was obtained as a white amorphous solid. The FAB-MS (positive-ion mode) showed the *quasi*-molecular ion peak $[M+1]^+$ at *m*/*z* 535. Its molecular formula was established as $C_{30}H_{46}O_8$ by HR-ESI-MS (*m*/*z* 557.3099 ($[M+Na]^+$; calc. 557.3090)), indicating eight degrees of unsaturation. The IR spectrum showed strong absorption bands at 3438 and 1769 cm^{-1}, indicating the presence of OH groups and of a γ-lactone moiety, respectively.



1 R^1 = Me, R^2 = OH
2 R^1 = OH, R^2 = Me
3 R^1 = H, R^2 = Me



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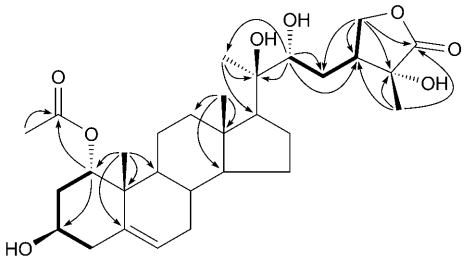
The ^{1}H-NMR spectrum of **1** (*Table*) displayed five Me *singlet*s at δ(H) 1.08, 1.15, 1.49, 1.73, and 2.14 (Me(19), Me(18), Me(21), Me(27), and Me(AcO)), one olefinic H-atom *doublet* at δ(H) 5.58 (H–C(6)) and two double *doublet*s at δ(H) 4.77 (*dd*, J = 9.0, 9.0) and 4.59 (*dd*, J = 9.0, 9.0) (CH_2(28), *ABX*-type pattern). The ^{13}C-NMR data indicated the presence of seven quaternary C-atoms, nine CH, nine CH_2, and five Me groups. Comparison of the NMR data of **1** with those of **3** indicated that **1** is also a perulactone-type withanolide, and that they possess a similar structure except for the presence of an OH group at C(25) in **1**. This assignment was supported by the 1D- and 2D-NMR spectra as shown in *Fig. 1*. In the ^{1}H-NMR spectrum, the signal for Me(27) (1.24 (*d*, J = 7.5)) in **3** was replaced by a *singlet* δ(H) 1.73 (*s*) in **1**, and this was also confirmed by HMBC correlations from Me(27) to C(24), C(25), and C(26), from CH_2(28) to C(23), C(24), C(25), and C(26), and the downfield shift of C(24) and C(25) (δ(C) +7.4 and 34.6 ppm, resp.). Additional key HMBCs were observed between Me(18) and C(12), C(13), and C(14), between Me(19) and C(1), C(5), C(9), and C(10), between Me(21) and C(17) and C(20), between H–C(22) and C(20), C(21), C(23), and C(24), and between H–C(1) and the AcO CO group. All these correlations firmly established the linkage of the above partial structural units (*Fig. 1*).



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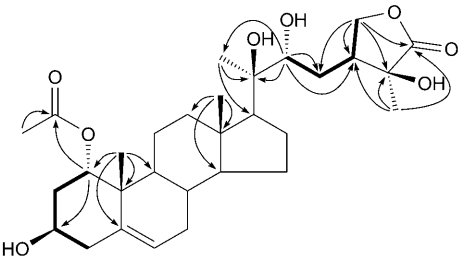

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Fig. 1. *Key* ^{1}*H,*^{1}*H-COSY* (—) *and HMBC* (→) *correlations for* **1** *and* **2**

The comparison of the H-atom coupling constants and other spectral data of **1** with those of **3** established the same relative configuration of compound **1** as that in **3** apart from the configuration at C(25). This was also confirmed by the ROESY spectrum

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. δ in ppm, in C_5D_5N, J in Hz

	1[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	5.34 (*s*)	75.9 (*d*)	5.34 (*s*)	75.7 (*d*)
CH_2(2)	2.05 (*m*, H_α),	36.5 (*t*)	2.06 (*m*, H_α),	36.5 (*t*)
	2.49 (br. *d*, J = 14.0, H_β)		2.49 (br. *d*, J = 13.6, H_β)	
H–C(3)	4.31–4.38 (*m*)	65.9 (*d*)	4.33–4.38 (*m*)	65.9 (*d*)
CH_2(4)	2.71–2.76 (*m*)	42.7 (*t*)	2.72–2.78 (*m*)	42.8 (*t*)
C(5)		138.7 (*s*)		138.6 (*s*)
H–C(6)	5.58 (*d*, J = 5.5)	123.8 (*d*)	5.58 (*d*, J = 4.8)	123.9 (*d*)
CH_2(7)	1.56–1.61 (*m*),	31.9 (*t*)	1.59–1.66 (*m*),	31.9 (*t*)
	1.88–1.95 (*m*)		1.89–1.95 (*m*)	
H–C(8)	1.47–1.55 (*m*)	31.5 (*d*)	1.46–1.53 (*m*)	31.5 (*d*)
H–C(9)	1.47–1.54 (*m*)	42.5 (*d*)	1.49–1.56 (*m*)	42.5 (*d*)
C(10)		40.9 (*s*)		40.9 (*s*)
CH_2(11)	1.47–1.53 (*m*)	20.8 (*t*)	1.49–1.54 (*m*)	20.8 (*t*)
CH_2(12)	1.12–1.18 (*m*),	40.6 (*t*)	1.17–1.23 (*m*),	40.6 (*t*)
	2.06–2.14 (*m*)		2.11–2.17 (*m*)	
C(13)		43.6 (*s*)		43.5 (*s*)
H–C(14)	0.84–0.93 (*m*)	56.8 (*d*)	0.79–0.86 (*m*)	56.6 (*d*)
CH_2(15)	1.17–1.24 (*m*),	24.5 (*t*)	1.13–1.20 (*m*),	24.5 (*t*)
	1.55–1.61 (*m*)		1.49–1.55 (*m*)	
CH_2(16)	1.82–1.88 (*m*),	22.5 (*t*)	1.65–1.73 (*m*),	22.5 (*t*)
	2.30–2.38 (*m*)		2.25–2.31 (*m*)	
H–C(17)	1.66–1.72 (*m*)	55.6 (*d*)	1.61–1.68 (*m*)	55.5 (*d*)
Me(18)	1.15 (*s*)	14.0 (*q*)	1.11 (*s*)	13.9 (*q*)
Me(19)	1.08 (*s*)	19.7 (*q*)	1.07 (*s*)	19.7 (*q*)
C(20)		76.8 (*s*)		76.5 (*s*)
Me(21)	1.49 (*s*)	20.8 (*q*)	1.51 (*s*)	21.0 (*q*)
H–C(22)	4.01 (br. *d*, J = 10.5)	75.7 (*d*)	3.96 (*d*, J = 10.4)	76.4 (*d*)
CH_2(23)	1.87–1.92 (*m*, H_α),	28.8 (*t*)	1.65–1.73 (*m*, H_α),	29.4 (*t*)
	2.32–2.37 (*m*, H_β)		2.23–2.29 (*m*, H_β)	
H–C(24)	2.63–2.69 (*m*)	46.3 (*d*)	3.05–3.12 (*m*)	47.5 (*d*)
C(25)		72.9 (*s*)		74.4 (*s*)
C(26)		179.7 (*s*)		181.1 (*s*)
Me(27)	1.73 (*s*)	23.2 (*q*)	1.60 (*s*)	19.2 (*q*)
CH_2(28)	4.59 (*dd*, J = 9.0, 9.0, H_α),	72.5 (*t*)	4.28 (*dd*, J = 10.0, 6.8, H_α),	71.4 (*t*)
	4.77 (*dd*, J = 9.0, 9.0, H_β)		4.91 (*dd*, J = 9.2, 8.0, H_β)	
AcO	2.14 (*s*)	170.3 (*s*),	2.12 (*s*)	170.3 (*s*),
		21.1 (*q*)		21.1 (*q*)

[a]) At 500/125 MHz. [b]) At 400/100 MHz

(*Fig. 2*). In the ROESY spectrum of **1**, correlations between H–C(24) and H–C(22), H_β–C(28), as well as Me(27) indicated that the OH group at C(25) has α-orientation, and the configuration at C(25) is (*R**). From these data, the structure of **1** was finally identified as (1α,3β,20*R**,22*R**,24*R**,25*R**)-3,20,22,25-tetrahydroxy-26-oxo-26,28-epoxyergost-5-en-1-yl acetate.

Compound **2**, named perulactone D, was obtained as a white amorphous solid. It was assigned the same molecular formula $C_{30}H_{46}O_8$ as **1** by HR-ESI-MS (*m*/*z* 557.3091

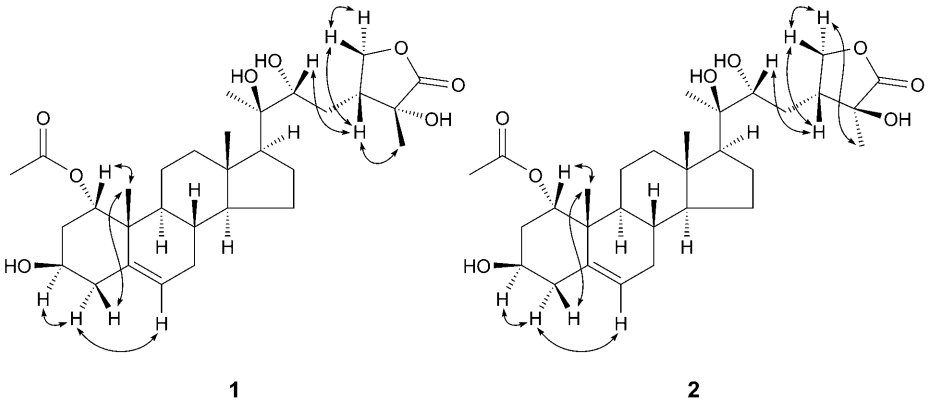

Fig. 2. *Key ROESY (↷) correlations for* **1** *and* **2**

([$M+\mathrm{Na}$]$^+$; calc. 557.3090)). The IR spectrum showed strong absorption bands at 3441 and 1774 cm^{-1}, indicating the presence of OH groups and of a γ-lactone moiety, respectively.

The ^{1}H- and ^{13}C-NMR spectra (*Table*) were very similar to those of **1**. The only difference between **2** and **1** was the configuration at C(25), which was confirmed by a ROESY experiment (*Fig. 2*). For **2**, a ROESY correlation of H_α–C(28) with Me(27) was observed, instead of the correlation of H–C(24) with Me(27) in **1**, suggesting that the OH group attached to C(25) has β-orientation and the configuration at C(25) is (*S**) in **2**. Consequently, perulactone D (**2**) was identified as the 25-epimer of perulactone C (**1**), and the structure of **2** was elucidated as (1α,3β,20*R**,22*R**, 24*R**,25*S**)-3,20,22,25-tetrahydroxy-26-oxo-26,28-epoxyergost-5-en-1-yl acetate.

The structures of the four known compounds were determined as perulactone (**3**) [5], perulactone B (**4**) [6], blumenol A [7], and (+)-(*S*)-dehydrovomifoliol [8] by comparison of their spectroscopic data with those reported in the literature. Blumenol A and (+)-(*S*)-dehydrovomifoliol were isolated from *P. peruviana* for the first time.

We are grateful to the Analytical Group of the Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, for the measurements of the spectra.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Co., Ltd*). TLC: silica-gel *G* plates; visualization by spraying with 10% H_2SO_4 in EtOH, followed by heating. Optical rotation: *Horiba-SEAP-300* spectropolarimeter. UV Spectra: *Shimadzu UV-2401PC* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr pellets; ν_{max} in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker AM-400* and *DRX-500* instruments; at 400/100 and 500/125 MHz, resp.; δ in ppm, *J* in Hz. FAB-MS: *VG AutoSpec-3000*. HR-ESI-MS: *API Qstar-Pulsar LC/TOF* mass spectrometers; in *m*/*z*.

Plant Material. The aerial parts of *P. peruviana* was collected in Kunming, Yunnan, P. R. China, in September 2005. A voucher specimen was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried aerial parts (6.0 kg) of *P. peruviana* were extracted with MeOH at r.t. (4 × 40 l). The extracts were combined and concentrated, and the residue was suspended in H_2O, and then successively partitioned with petroleum ether (PE), $CHCl_3$, and BuOH, resp. The $CHCl_3$-soluble extract (33 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100 : 0 → 80 : 20) to afford 11 fractions: *Fr. 1–11*. Compounds **1** (17 mg), **2** (12 mg), and **3** (24 mg) were obtained from *Fr. 8*. Compound **4** (172 mg) was obtained from *Fr. 7*. Blumenol A (13 mg) and (+)-(*S*)-dehydrovomifoliol (10 mg) were obtained from *Fr. 4* after repeated CC.

Perulactone C (= *(1α,3β,20*R*,22*R*,24*R*,25*R*)-3,20,22,25-Tetrahydroxy-26-oxo-26,28-epoxyergost-5-en-1-yl Acetate*; **1**). White amorphous solid. UV (MeOH): 204 (3.58). $[\alpha]_D^{24.8} = 0.0$ ($c = 0.09$, MeOH). IR (KBr): 3438, 2968, 2941, 1769, 1734, 1714, 1634. ^{1}H- and ^{13}C-NMR: *Table*. FAB-MS (glycerol; pos.): 627 ($[M + \text{glyc.} + H]^+$), 535 ($[M + H]^+$). HR-ESI-MS (pos.): 557.3099 ($C_{30}H_{46}NaO_8^+$, $[M + Na]^+$; calc. 557.3090).

Perulactone D (= *(1α,3β,20*R*,22*R*,24*R*,25*S*)-3,20,22,25-Tetrahydroxy-26-oxo-26,28-epoxyergost-5-en-1-yl Acetate*; **2**). White amorphous solid. UV (MeOH): 203 (3.45). $[\alpha]_D^{25.3} = 0.0$ ($c = 0.12$, MeOH). IR(KBr): 3441, 2948, 2939, 1774, 1735, 1713, 1631. ^{1}H- and ^{13}C-NMR: *Table*. FAB-MS (glycerol; pos.): 720 ($[M + 2\ \text{glyc.}]^+$), 628 ($[M + \text{glyc.} + 2\ H]^+$), 535 ($[M + H]^+$). HR-ESI-MS (pos.): 557.3091 ($C_{30}H_{46}NaO_8^+$, $[M + Na]^+$); calc. 557.3090).

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Received January 6, 2009