Steroids 77 (2012) 36–44

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Ten new withanolides from *Physalis peruviana*

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ARTICLE INFO

Article history:
Received 23 August 2010
Received in revised form 25 September 2011
Accepted 27 September 2011
Available online 10 October 2011

Keywords:
Physalis peruviana
Withanolides
Perulactones E–H
Withaperuvins I–N

ABSTRACT

Ten new withanolides, including four perulactone-type withanolides, perulactones E–H (**1–4**), three 28-hydroxy-withanolides, withaperuvins I–K (**5–7**), and three other withanolides, withaperuvins L–N (**8–10**), together with six known compounds (**11–16**) were isolated from the aerial parts of *Physalis peruviana*. The structures of these compounds were elucidated on the basis of extensive spectroscopic analyses (1D and 2D NMR, IR, HR-MS) and chemical methods.

1. Introduction

Withanolides are a group of naturally occurring C_{28} steroidal lactones that contain an intact or modified ergostane skeleton. These compounds are produced mainly by the Solanaceae family, in particular by the genera *Withania*, *Physalis*, *Datura*, *Nicandra*, *Dunalia*, *Lycium*, *Tubocapsicum* and *Jaborosa* [1]. In addition, withanolides often display significant pharmacological activities, including antimicrobial, antitumor, anti-inflammatory, hepatoprotective, immunomodulatory, antibacterial, insect-antifeedant, and insect-repellent activities [1]. Perulactones are a small group of withanolides in which C-28 and C-26 are oxidized to form a γ-lactone ring, and 28-hydroxy-withanolides may be their precursors. Thus far, only four perulactone-type withanolides have been isolated from the genus *Physalis* [2–4].

The genus *Physalis* includes approximately 120 species distributed mainly in South and North America. Five species of *Physalis* are found in China [5]. *Physalis peruviana* (Chinese name: deng-long-guo) is cultivated in China for its edible fruits. It is also used in folk medicine for treating diseases such as cancer, malaria, asthma, hepatitis, dermatitis, and rheumatism [6]. In our previous investigations, two new perulactone-type withanolides, perulactones C and D [4], and a novel 1,10-seco withanolide, 1,10-seco withaperuvin C [7], were isolated from the aerial parts of *P. peruviana*. As part of our continuing search for novel withanolides from this plant, we further isolated ten new withanolides, including four other new perulactones, perulactones E–H (**1–4**), three new 28-hydroxy-withanolides, withaperuvins I–K (**5–7**), and another three new withanolides, withaperuvins L–N (**8–10**), together with six known withanolides, phyperunolides A (**11**) and B (**12**) [8], withanolide S 5-methyl ether (**13**) [9,10], withanolide C (**14**) [11], withanolide S (**15**) [12,13] and physalactone (**16**) [14] (Fig. 1), from the aerial parts of *P. peruviana*. This report describes the structure elucidation of the new compounds on the basis of spectroscopic analyses and chemical methods.

2. Experimental procedures

2.1. General

Optical rotations were determined on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer. UV spectra were obtained on a Shimadzu UV-2401PC spectrometer. NMR spectra were recorded in $CDCl_3$ and C_5D_5N using TMS as an internal standard on Bruker AM-400, DRX-500, and AVANCE III-600 spectrometers. MS data were obtained on a VG Auto Spec-3000 or an API QSTAR Pulsar spectrometer. TLC was performed on silica gel G and viewed by spraying with 10% H_2SO_4 in EtOH, followed by heating. Silica gel (200–300 mesh) for column chromatography (CC) was obtained from Qingdao Marine Chemical Company, Qingdao, China.

2.2. Plant material

The aerial parts of *P. peruviana* were collected from Kunming, Yunnan Province, China, in September 2005, and were identified by Professor Chengmin Zhang, Kunming Institute of Botany. A

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doi:10.1016/j.steroids.2011.09.011

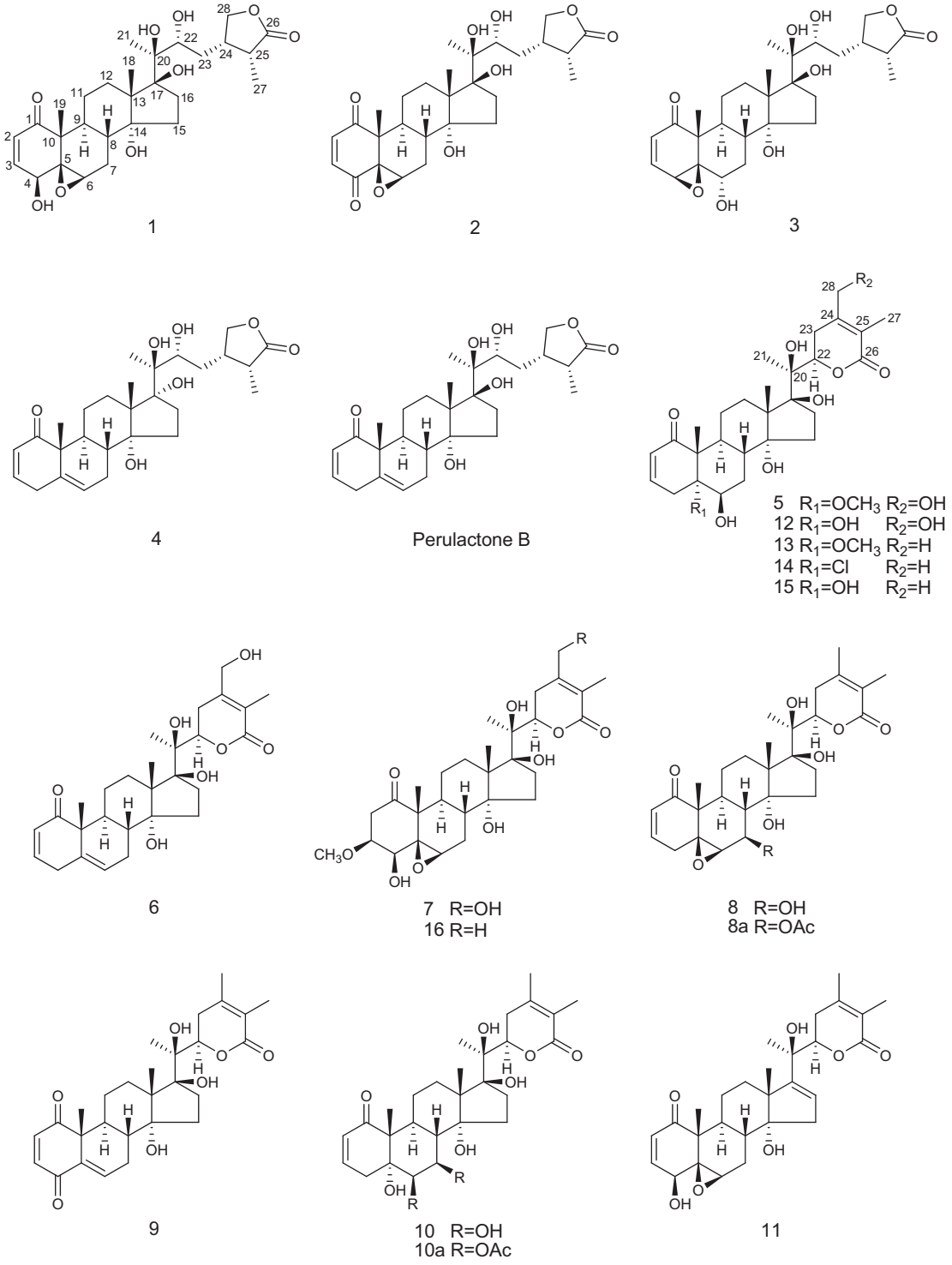


Fig. 1. Structures of compounds **1**–**16**, **8a**, **10a** and Perulactone B.

voucher specimen (No. 20050911) is deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried aerial parts of *P. peruviana* (6 kg) were extracted with MeOH (4 × 40 L) at room temperature. The crude extracts were combined and concentrated. The residue was suspended in H_2O and successively partitioned with petroleum ether (PE), $CHCl_3$ and *n*-BuOH. The $CHCl_3$ portion of the extract (33 g) was subjected to column chromatography (CC) on silica gel and eluted with $CHCl_3$/MeOH (1:0–4:1, v/v) to give seven factions (fractions A–G). Fraction C (13.7 g, $CHCl_3$/MeOH 30:1) was chromatographed over silica gel with PE/acetone (10:1 to 1:1, v/v) to give 11 subfractions (C1–C11). Subfraction C6 was further purified by successive silica-gel CC with $CHCl_3$/MeOH (20:1, v/v) and $CHCl_3$/acetone (7:3, v/v) to afford **4** (9 mg). Subfraction C7 was

subjected to successive CC on silica gel ((1) PE/acetone (3:1 to 1:1, v/v); (2) PE/EtOAc (3:7, v/v); (3) $CHCl_3$/acetone (7:3, v/v); and (4) PE/acetone (1:1, v/v)) to yield **1** (9 mg), **2** (7 mg) and **3** (14 mg). Subfraction C9 was purified by CC on silica gel and eluted with PE/EtOAc (10:1–1:3, v/v), $CHCl_3$/acetone (4:1, v/v) and PE/acetone (7:3, v/v), respectively, to give **6** (20 mg), **11** (10 mg) and **16** (14 mg). Fraction D (6.2 g, $CHCl_3$/MeOH 15:1) was purified by CC and eluted with $CHCl_3$/acetone (10:1–1:1, v/v) to afford six subfractions (D1−D6). Subfraction D1 was separated using CC with PE/acetone (7:3, v/v) to afford **9** (8 mg). Fraction D3 was chromatographed over a silica-gel column (PE/acetone 10:1–1:3, v/v) to afford **5** (13 mg) and crude crystals, which were washed with acetone to give pure **12** (23 mg). Fraction D4 was purified by CC with PE/acetone (1:1, v/v) to give **8** (102 mg). Compound **10** (25 mg) was obtained from fraction D5 after purification by a silica-gel CC using $CHCl_3$/MeOH (9:1, v/v). Fraction E (5.4 g, $CHCl_3$/MeOH 10:1) was subjected to repeated CC with PE/acetone (10:1 to 1:1, v/v), $CHCl_3$/MeOH (20:1 to 9:1, v/v) and $CHCl_3$/acetone (3:2 to 1:1, v/v) to afford **7** (16 mg), **13** (23 mg), **14** (146 mg) and **15** (7 mg).

2.3.1. Perulactone E (**1**)

$C_{28}H_{40}O_9$; white amorphous solid; $[\alpha]_D^{14.5}$ = +144.4° (*c* = 0.31, MeOH); UV (MeOH) λ_{max} (log ε): 215 (3.81) nm; IR (KBr) ν_{max}: 3441, 2977, 2950, 1757, 1676, 1384, 1251, 1017 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS (positive) *m/z*: 1063 $[2M+Na]^+$, 543 $[M+Na]^+$; HRESI-MS [(positive) *m/z*: 543.2585 $[M+Na]^+$ (calcd. for $C_{28}H_{40}O_9Na$, 543.2570).

2.3.2. Perulactone F (**2**)

$C_{28}H_{38}O_9$; white amorphous solid; $[\alpha]_D^{14.3}$ = +172.2° (*c* = 0.31, MeOH); UV (MeOH) λ_{max} (log ε): 224 (4.03) nm; IR (KBr) ν_{max}: 3565, 3450, 3258, 3090, 2972, 2942, 2868, 1776, 1687, 1368, 1280, 1195, 1049, 1006 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS (positive) *m/z*: 1059 $[2M+Na]^+$, 541 $[M+Na]^+$; HRESI-MS (positive) *m/z*: 541.2413 $[M+Na]^+$ (calcd. for $C_{28}H_{38}O_9Na$, 541.2413).

2.3.3. Perulactone G (**3**)

$C_{28}H_{40}O_9$; colorless amorphous solid; $[\alpha]_D^{14.4}$ = +40.9° (*c* = 0.21, MeOH); UV (MeOH) λ_{max} (log ε): 236 (3.19), 225 (3.20) nm; IR (KBr) ν_{max}: 3432, 2946, 1756, 1670, 1633, 1382, 1048, 1005 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS (positive) *m/z*: 1063 $[2\,M+Na]^+$, 543 $[M+Na]^+$; HRESI-MS (positive) *m/z*: 543.2583 $[M+Na]^+$ (calcd. for $C_{28}H_{40}O_9Na$, 543.2570).

2.3.4. Perulactone H (**4**)

$C_{28}H_{40}O_7$; colorless amorphous solid; $[\alpha]_D^{12.4}$ = +8.2° (*c* = 0.19, MeOH); UV (MeOH) λ_{max} (log ε): 222 (3.65) nm; IR (KBr) ν_{max}: 3431, 2970, 2922, 1751, 1662, 1384, 1044, 1007 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESI-MS (positive) *m/z*: 999 $[2M+Na]^+$, 511 $[M+Na]^+$; HRESI-MS (positive) *m/z*: 511.2672 $[M+Na]^+$ (calcd. for $C_{28}H_{40}O_7Na$, 511.2671).

2.3.5. Withaperuvin I (**5**)

$C_{29}H_{42}O_9$; colorless amorphous solid; $[\alpha]_D^{21.2}$ = +99.3° (*c* = 0.41, MeOH); UV (MeOH) λ_{max} (log ε): 222 (3.84) nm; IR(KBr) ν_{max}:

Table 1
^1H and ^{13}C NMR data (500/125 MHz) of compounds **1**–**3**[a] (δ in ppm, *J* values in Hz).

Position	**1** (C_5D_5N)		**2** ($CDCl_3$)		**3** (C_5D_5N)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		202.7		202.1		200.0
2	6.44 d (10.0)	132.6	6.84 d (10.3)	141.6	6.24 d (10.0)	130.4
3	7.20[b]	144.9	6.89 d (10.3)	139.3	7.09 dd (10.0, 4.0)	143.9
4	4.03 d (6.4)	70.5		193.9	4.21 d (4.0)	49.2
5		64.7		64.1		69.6
6	3.36 brs	61.0	3.49 br.s	64.2	4.58 dd (10.4, 4.8)	64.9
7	2.41 m, 2.21 m	26.8	2.08 m, 1.93 m	25.7	2.45 m, 2.20 m	35.7
8	2.15 m	34.9	2.01 m	33.8	2.21 m	38.5
9	2.06 m	37.9	2.33 m	36.0	2.45 m	41.5
10		48.7		49.8		52.1
11	1.91 m	21.8	2.09 m,1.61 m	23.0	1.71 m, 1.18 m	23.4
12	2.61 m, 1.69 m	30.5	2.32 m, 1.51 m	29.8	2.54 m, 1.99 m	30.7
13		54.8		54.2		55.1
14		82.4		82.7		82.8
15	1.78 m	32.9	1.73 m, 1.56 m	32.5	1.93 m, 1.85 m	32.9
16	2.95 m, 1.93 m	37.3	2.67 m, 1.48 m	37.5	2.97 m, 1.94 m	37.3
17		88.4		88.2		88.4
18	1.34 s	21.0	1.10 s	20.6	1.38 s	21.5
19	1.93 s	17.0	1.39 s	19.3	1.61 s	12.6
20		79.6		78.6		79.4
21	1.65 s	19.6	1.26 s	19.2	1.64 s	19.6
22	4.44 d (10.0)	72.9	4.04 d (9.6)	72.4	4.41 d (10.4)	72.9
23	2.69 m, 1.66 m	32.6	2.19 m, 1.36 m	31.8	2.69 m, 1.65 m	32.6
24	3.04 m	39.2	2.76 m	38.1	3.06 m	39.2
25	2.81 m	38.3	2.67 m	37.7	2.83 m	38.3
26		180.9		180.7		180.9
27	1.33 d (7.6)	10.8	1.19 d (7.5)	10.5	1.34 d (7.6)	10.8
28a	4.50 dd (8.6, 7.2)	72.8	4.40 dd (9.0, 7.0)	72.4	4.52 dd (8.4, 7.2)	72.8
28b	4.34 dd (8.4, 8.4)		4.09 dd (8.8, 8.4)		4.37 dd (8.4, 8.4)	

[a] Assignments were based on 2D NMR spectra.
[b] Signal overlapped with solvent signal.

3424, 2951, 2930, 1692, 1384, 1084, 1003, 953 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; ESI-MS (positive) *m/z*: 1091 $[2M+Na]^+$, 557 $[M+Na]^+$; HRESI-MS (positive) *m/z*: 557.2751 $[M+Na]^+$ (calcd. for $C_{29}H_{42}O_9Na$, 557.2726).

2.3.6. Withaperuvin J (**6**)

$C_{28}H_{38}O_7$; colorless amorphous solid; $[\alpha]_D^{14.3} = +48.6^\circ$ (*c* = 0.17, MeOH); UV (MeOH) λ_{max} (log ε): 222 (3.81) nm; IR (KBr) ν_{max}: 3428, 2955, 2928, 1685, 1666, 1383, 1126, 1003 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; ESI-MS (positive) *m/z*: 995 $[2M+Na]^+$, 509 $[M+Na]^+$; HRESI-MS (positive) *m/z*: 509.2512 $[M+Na]^+$ (calcd. for $C_{28}H_{38}O_7Na$, 509.2515).

2.3.7. Withaperuvin K (**7**)

$C_{29}H_{42}O_{10}$; colorless amorphous solid; $[\alpha]_D^{13.8} = +8.1^\circ$ (*c* = 0.38, MeOH); UV (MeOH) λ_{max} (log ε): 224 (3.70) nm; IR (KBr) ν_{max}: 3423, 2953, 1701, 1376, 1091, 1016, 953 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; ESI-MS (positive) *m/z*: 1123 $[2M+Na]^+$, 573 $[M+Na]^+$; HRESI-MS (positive) *m/z*: 573.2675 (calcd. for $C_{29}H_{42}O_{10}Na$, 573.2675).

2.3.8. Withaperuvin L (**8**)

$C_{28}H_{38}O_8$; colorless amorphous solid; $[\alpha]_D^{20.8} = +99.8^\circ$ (*c* = 0.34, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 240 (3.56) nm; IR(KBr) ν_{max}: 3429, 2973, 2952, 1679, 1385, 1139, 1088, 1019, 967 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 4 and 5; ESI-MS (positive) *m/z*: 1027 $[2M+Na]^+$, 525 $[M+Na]^+$; HRESI-MS (positive) *m/z*: 525.2470 $[M+Na]^+$ (calcd. for $C_{28}H_{38}O_8Na$, 525.2464).

2.3.9. Acetylation of compound **8**

Treatment of compound **8** (18 mg) with Ac_2O (1 ml) in pyridine (1 ml) at room temperature for 24 h afforded monoacetate **8a** (12 mg). ^1H NMR (600 MHz, $CDCl_3$): δ_H 6.57 (1H, ddd, *J* = 10.0, 6.4, 3.2 Hz, H-3), 5.98 (1H, dd, *J* = 10.0, 2.8 Hz, H-2), 5.37 (1H, dd, *J* = 9.4, 1.0 Hz, H-7), 4.84 (1H, dd, *J* = 12.4, 4.2 Hz, H-22), 3.27 (1H, d, *J* = 1.3 Hz, H-6), 2.02 (3H, s, 7-OAc), 1.87 (3H, s, Me-28), 1.81 (3H, s, Me-27), 1.34 (3H, s, Me-21), 1.19 (3H, s, Me-19), 1.04 (3H, s, Me-18); ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 5.

2.3.10. Withaperuvin M (**9**)

$C_{28}H_{36}O_7$; light-yellow solid; $[\alpha]_D^{14.7} = +116.4^\circ$ (*c* = 0.23, MeOH); UV (MeOH) λ_{max} (log ε): 225 (4.21) nm; IR (KBr) ν_{max}: 3429, 2927, 1691, 1626, 1385, 1135, 1090, 1002 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 4 and 5; EI-MS *m/z*: 484 $[M]^+$, 466 $[M-H_2O]^+$, 448 $[M-2H_2O]^+$, 169, 152, 125,109; HREI-MS *m/z*: 484.2455 $[M]^+$ (calcd. for $C_{28}H_{36}O_7$, 484.2461).

2.3.11. Withaperuvin N (**10**)

$C_{28}H_{40}O_9$; white amorphous solid; $[\alpha]_D^{14.8} = +125.2^\circ$ (*c* = 0.23, pyridine); UV (MeOH) λ_{max} (log ε): 224 (3.82) nm; IR(KBr) ν_{max}: 3518, 3421, 2926, 1684, 1389, 1143, 1012, 964 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 4 and 5; ESI-MS (positive) *m/z*: 1063

Table 2
^1H and ^{13}C NMR data of compounds **4**[a] and Perulactone B (δ in ppm, *J* values in Hz).

Position	**4** (C_5D_5N)[b]		**4** ($CDCl_3$)[d]		Perulactone B (C_5D_5N)[b]		Perulactone B ($CDCl_3$)[c]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		203.9		205.0		204.2		204.7
2	5.99 dd (10.0, 2.5)	128.0	5.81 dd (10.0, 2.1)	127.8	5.99 dd (10.0, 2.0)	128.1	5.87 dd (10.0, 2.2)	127.5
3	6.68 ddd (10.0, 5.0, 2.5)	145.7	6.57 ddd (10.0, 4.8, 2.4)	146.1	6.69 ddd (10.0, 4.8, 2.4)	145.8	6.80 ddd (10.0, 4.7, 2.4)	146.1
4	3.22 brd (21.5)	33.7	3,23 brd (21.3)	33.6		33.7		33.4
	2.72 m		2.80 dd (21.3, 4.9)					
5		135.9		135.3		135.9		135.6
6	5.56 d (5.5)	125.2	5.55 d (5.0)	124.9	5.58 (4.2)	125.6	5.62 d (4.4)	124.9
7	2.40 m,	25.5	1.96 m,	24.9		26.2		25.5
	1.89 m		1.78 m					
8	2.76 m	36.6	1.79 m	36.3		36.5		35.9
9	1.95 m	36.8	2.14 td (12.0, 4.7)	35.9		37.7		37.0
10		51.3		50.9		51.3		50.9
11	2.66 m,	22.8	2.22 m, 1.54 m	22.0		23.9		23.0
	1.83 m							
12	2.79 m,	27.4	2.27 td (13.1, 4.2)	26.3		33.0		32.5
	1.83 m		1.43 m					
13		51.6		50.1		54.9		54.4
14		86.3		86.2		82.7		83.9
15	2.05 m, 1,78 m	33.5	1.73 m,	32.8		32.6		31.6
			1.51 m					
16	3.16 m, 2,12 m	34.3	2.50 dd (14.4, 10.8)	33.9		37.2		37.6
			1.78 m					
17		90.7		92.2		88.7		88.2
18	1.41 s	19.1	0.91 s	19.2	1.42 s	21.4	1.15 s	20.6
19	1.27 s	19.1	1.20 s	19.1	1.28 s	19.7	1.25 s	19.0
20		77.3		76.8		79.3		79.4
21	1.64 s	21.5	1.20 s	23.3	1.65 s	19.0	1.27 s	18.9
22	4.03 d (10.0)	77.4	3.66 d (9.7)	76.6	4.45 d (10.4)	73.1	4.16 d (10.0)	72.6
23	2.51 m,	31.7	1.74 m,	29.4		31.3		30.1
	1.71 m		1.57 m					
24	2.96 m	38.8	2.70 m	37.0		39.2		38.1
25	2.73 m	38.2	2.63 m	38.0		38.3		37.9
26		180.7		181.6		180.9		181.0
27	1.22 d (7.5)	10.7	1.13 d (7.5)	10.7	1.32 d (7.5)	10.8	1.12 d (7.0)	10.6
28a	4.47 dd (8.0, 7.5)	72.7	4.40 dd (9.0, 7.3)	73.1	4.51 dd (8.8, 7.2)	72.9	4.36 dd (8.5, 7.0)	73.3
28b	4.29 dd (8.5, 8.5)		4.04 dd (8.8, 8.8)		4.35 dd (8.4, 8.4)		4.03 dd (8.5, 8.5)	

[a] Assignments were based on 2D NMR spectra.
[b] Spectra were recorded at 500/125 MHz.
[c] Spectra were recorded at 400/100 MHz.
[d] Spectra were recorded at 600/150 MHz; a few drops of CD_3OD were added to improve solubility.

Table 3
^{1}H and ^{13}C NMR data of compounds **5**–**7**[a] (δ in ppm, J values in Hz).

Position	**5** (C_5D_5N)[c]		**6** ($CDCl_3$)[c]		**7** (C_5D_5N)[b]		**7** ($CDCl_3$)[d]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		204.5		204.5		209.8		211.3
2	6.01 dd (10.0, 2.4)	129.6	5.84 dd (10.0, 2.0)	128.0	3.10 m	41.2	2.73 dd (15.4, 7.8) 2.61 dd (15.4, 3.8)	40.3
3	6.51 dd (10.0, 3.6)	140.1	6.77 ddd (10.0, 4.8, 2.4)	145.4	3.92 m	78.9	3.56 m	78.7
4	3.36 brd (20.0)2.46 dd (20.0, 5.2)	28.3	3.26 brd (21.2) 2.83 dd (21.2, 4.8)	33.5	3.91 m	75.0	3.36 d (2.5)	74.7
5		82.8		135.0		65.2		65.0
6	4.27 brs	68.5	5.60 brs	125.3	3.53 s	59.1	3.21 s	59.7
7	2.59 m, 1.92 m	30.5	2.71 m, 1.84 m	25.6	2.62 m, 2.25 m	26.7	1.98 m	25.8
8	2.66 m	34.8	1.83 m	37.3	2.03 m	34.8	1.68 m	33.9
9	3.41 m	34.6	2.33 m	35.6	2.30 m	36.8	1.69 m	35.9
10		53.6		50.7		51.1		50.6
11	2.78 m, 1.71 m	23.6	2.28 m, 1.62 m	23.1	1.60 m, 1.31 m	21.8	1.35 m, 1.08 m	20.9
12	2.97 m, 1.68 m	31.9	2.43 m, 1.42 m	30.2	2.60 m, 1.48 m	30.2	2.14 m, 1.17 m	29.3
13		55.6		54.5		55.1		54.4
14		83.3		82.8		81.9		82.1
15	1.89 m	33.4	1.69 m, 1.57 m	32.4	1.77 m	33.1	1.59 m 1.49 dd (11.8, 9.1)	32.3
16	3.13 m, 1.96 m	37.5	2.69 m, 1.53 m	37.3	3.05 m, 1.96 m	37.4	2.55 m 1.42 dd (14.7, 8.6)	37.2
17		88.7		88.1		88.4		87.7
18	1.46 s	21.8	1.12 s	20.4	1.28 s	20.7	0.93 s	20.0
19	1.66 s	16.4	1.22 s	18.9	1.74 s	15.2	1.18 s	14.3
20		79.5		79.1		79.5		78.7
21	1.84 s	20.0	1.42 s	20.5	1.81 s	19.7	1.31 s	19.1
22	5.39 dd (13.2, 2.4)	83.1	4.89 d (11.2)	81.5	5.33 dd (13.5, 2.5)	82.9	4.74 dd (13.4, 2.3)	81.9
23	3.74 brd (18.4) 2.77 m	30.2	2.90 brd (18.0) 2.47 m	28.4	3.71 brd (18.0) 2.75 m	30.1	2.84 brd (17.5) 2.29 t (15.7)	28.9
24		154.6		152..7		154.9		153.5
25		121.0		121.3		121.0		121.1
26		167.4		166.5		167.4		167.8
27	2.01 s	12.1	1.85 s	11.8	2.03 s	12.1	1.77 s	11.7
28	4.65 d (14.4) 4.36 d (14.4)	61.0	4.39 d (14.4) 4.28 d (14.4)	61.3	4.71 d (14.5) 4.41 d (14.5)	61.1	4.28 d (14.5) 4.14 d (14.5)	61.0
-OCH_3	2.97 s	49.7			3.26 s	56.7	3.25 s	56.9

[a] Assignments were based on 2D NMR spectra.
[b] Spectra were recorded at 500/125 MHz.
[c] Spectra were recorded at 400/100 MHz.
[d] Spectra were recorded at 600/150 MHz; a few drops of CD_3OD were added to improve solubility.

$[2M+Na]^+$, 543 $[M+Na]^+$; HRESI-MS (positive) *m/z*: 543.2557 $[M+Na]^+$ (calcd. for $C_{28}H_{40}O_9Na$, 543.2570).

*2.3.12. Acetylation of compound **10***

Treatment of compound **10** (10 mg) with Ac_2O (1 ml) in pyridine (1 ml) at room temperature for 24 h afforded diacetate **10a** (7 mg). ^{1}H NMR (600 MHz, C_5D_5N): δ_H 6.58 (1H, dd, J = 11.0, 3.1 Hz, H-7), 6.55 (1H, dd, J = 10.1, 3.6 Hz, H-3), 6.15 (1H, d, J = 10.1 Hz, H-2), 5.90 (1H, d, J = 3.1 Hz, H-6), 5.32 (1H, dd, J = 13.3, 2.4 Hz, H-22), 2.21 (3H, s, 6-OAc), 2.09 (3H, s, 7-OAc), 1.97 (3H, s, Me-27), 1.83 (3H, s, Me-21), 1.72 (3H, s, Me-28), 1.61 (3H, s, Me-18), 1.55 (3H, s, Me-19); ^{13}C NMR (150 MHz, C_5D_5N), see Table 5.

3. Results and discussion

The MeOH extract of the aerial parts of *P. peruviana* was successively partitioned with petroleum ether (PE), $CHCl_3$, and *n*-BuOH. The column chromatography of the $CHCl_3$-soluble extract (33 g) yielded seven fractions. Repeated column chromatography of these fractions yielded ten new withanolides **1**–**10** and six known compounds (**11**–**16**).

Perulactone E (**1**) was obtained as a white amorphous solid; its molecular formula was determined as $C_{28}H_{40}O_9$ by positive HRESI-MS (*m/z* 543.2585 $[M+Na]^+$, calcd. for 543.2570), which indicates nine degrees of unsaturation. The IR spectrum showed the presence of hydroxyl (3441 cm^{-1}), α,β-unsaturated ketone (1676 cm^{-1}), and saturated-γ-lactone (1757 cm^{-1}) groups. The ^{1}H NMR (Table 1) spectrum of **1** displayed two olefinic protons [δ_H 6.44 (1H, d, J = 10.0 Hz, H-2) and 7.20 (1H, overlapped with solvent signal, H-3)], three oxymethine protons [δ_H 3.36 (1H, brs (broad singlet), H-6), 4.03 (1H, d, J = 6.4 Hz, H-4) and 4.44 (1H, d, J = 10.0 Hz, H-22)], an oxymethylene group [δ_H 4.50 (1H, dd, J = 8.6, 7.2 Hz, H-28a) and 4.34 (1H, dd, J = 8.4, 8.4 Hz, H-28b)], and four methyl groups [δ_H 1.33 (3H, d, J = 7.6 Hz, Me-27), 1.34 (3H, s, Me-18), 1.65 (3H, s, Me-21) and 1.93 (3H, s, Me-19)]. The ^{13}C NMR and DEPT (Table 1) spectra showed 28 carbon signals, including four methyl, seven methylene, nine methine, and eight quaternary carbons. Detailed comparison of the ^{1}H and ^{13}C NMR spectral data of **1** with those of a known withanolide, perulactone B [3], which was obtained in our previous work from this plant [4], indicated that **1** possesses the same perulactone skeleton and that **1** has the same substituent patterns and relative configuration in rings C-D and the side chain as perulactone B. The differences between these two compounds were the functional groups in rings A and B. In the ^{1}H,^{1}H-COSY spectrum of **1** (Fig. 2), H-3 correlated with H-2 and the oxygenated methine proton H-4 indicated the presence of a 4β-hydroxy-2-en-1-one unit in ring A [15]. The broad singlet signal of H-6 (δ_H 3.36) and the signals for two oxygenated carbons at δ_C 64.7 (C, C-5) and 61.0 (CH, C-6) in the ^{13}C NMR spectrum suggested a 5β,6β-epoxy group in **1** [15]. These assignments

Table 4
^{1}H NMR data of compounds **8**–**10**[a] (δ in ppm, *J* values in Hz).

Position	**8** ($CDCl_3$)[b]	**8** (C_5D_5N)[c]	**9** ($CDCl_3$)[b]	**10** (C_5D_5N)[b]
2	6.02 dd (10.0, 2.7)	6.17 dd (10.0, 2.7)	6.64 d (10.3)	6.12 dd (10.0, 2.4)
3	6.82 ddd(10.0, 6.0, 2.0)	6.77 m	6.70 d (10.3)	6.61 ddd (10.0, 4.6, 1.7)
4	2.92 brd (18.5),	3.00 brd (18.4)		3.76 m, 2.44 m
	1.90 m	1.95 dd (18.4, 6.4)		
6	3.21 brs	3.57 brs	6.89 dd (5.6, 2.4)	4.23 d (3.2)
7	4.18 d (8.9)	4.82 d (9.1)	2.47 m, 2.14 m	5.20 dd (10.4, 3.2)
8	1.82 m	2.41 m	1.89 m	2.70 m
9	1.82 m	2.56 td (12.1, 4.9)	2.55 m	3.73 m
11	2.00 m,	2.35 m,	2.36 m,	2.97 m,
	1.58 m	1.81 m	1.55 m	1.86 m
12	2.24 m, 1.26 m	2.79 m, 1.51 brd (10.4)	2.53 m,	3.07 m,
			1.72 m	1.64 m
15	1.95 m	2.79 m,	1.72 m,	2.85 m,
		2.41 m	1.60 m	2.47 m
16	2.67 m, 1.45 m	3.15 m,	2.74 m,	3.20 m,
		2.03 m	1.46 m	2.02 m
18	1.09 s	1.39 s	1.13 s	1.55 s
19	1.23 s	1.45 s	1.38 s	1.75 s
21	1.40 s	1.81 s	1.44 s	1.82 s
22	4.87 dd (11.5, 5.0)	5.31 dd (13.5, 2.8)	4.92 dd (10.9, 5.6)	5.34 dd(13.3, 2.8)
23	2.49 m	2.96 m,	2.53 m	2.97 m,
		2.70 m		2.71 m
27	1.86 s	1.97 s	1.88 s	1.94 s
28	1.93 s	1.75 s	1.94 s	1.69 s

[a] Assignments were based on 2D NMR spectra.
[b] Spectra were recorded at 500 MHz.
[c] Spectra were recorded at 600 MHz.

Table 5
^{13}C NMR data of compounds **8**–**10**, **8a** and **10a**[a].

Position	**8** ($CDCl_3$)[b]	**8** (C_5D_5N)[c]	**8a** ($CDCl_3$)[c]	**9** ($CDCl_3$)[b]	**10** (C_5D_5N)[b]	**10a** (C_5D_5N)[c]
1	202.2	203.4	202.1	202.0	204.9	203.9
2	129.9	130.3	130.1	139.1	129.4	129.9
3	143.3	144.9	143.4	140.3	141.4	140.8
4	32.4	33.3	32.4	187.9	37.3	36.2
5	65.7	66.0	65.4	138.8	78.7	77.3
6	67.6	69.4	64.2	139.1	79.4	76.3
7	67.8	68.6	71.2	26.3	68.3	71.1
8	42.2	43.2	39.3	36.0	43.1	41.2
9	36.9	38.4	36.7	35.4	34.5	35.1
10	47.9	49.0	47.9	51.4	52.4	52.3
11	22.6	24.2	22.8	22.1	23.6	23.9
12	29.7	31.5	29.8	30.1	32.1	32.2
13	55.1	56.2	55.2	54.6	56.5	56.8
14	81.8	82.7	80.9	81.5	83.3	82.1
15	35.5	37.1	35.2	32.4	37.0	37.0
16	38.2	38.4	38.2	37.8	38.1	38.0
17	86.8	87.7	86.6	87.9	87.6	87.6
18	20.5	21.7	20.7	20.6	22.0	22.6
19	14.1	15.3	14.6	23.6	16.4	16.2
20	79.0	79.9	79.3	79.1	79.5	80.0
21	19.5	20.2	19.9	19.8	20.0	20.2
22	80.2	82.3	79.7	79.7	82.2	82.2
23	34.2	35.7	34.4	34.3	35.4	35.7
24	150.7	151.5	150.9	150.5	150.7	151.2
25	121.4	122.0	121.6	121.5	121.6	122.0
26	166.4	167.5	166.1	165.9	167.0	167.4
27	12.3	13.1	12.6	12.4	12.6	13.1
28	20.5	20.7	20.9	20.6	20.2	20.7
6-OAc						171.1
						21.4
7-OAc			170.9, 21.9			170.7
						22.1

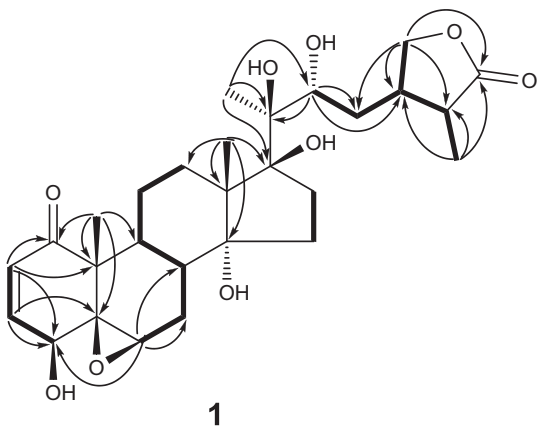
[a] Assignments were based on 2D NMR spectra.
[b] Spectra were recorded at 125 MHz.
[c] Spectra were recorded at 150 MHz.

were further confirmed by HMBC and ROESY experiments (Figs. 2 and 3). The ^{13}C NMR spectrum showed signals at δ_C 82.4 (C-14) and 88.4 (C-17), which were assigned to two oxygenated quaternary carbons. The chemical shifts of C-13, C-15, and C-16 (δ_C 54.8, 32.9, and 37.3, respectively) agreed with those reported for withanolides with 14α-OH and 17β-OH groups [3,16]. The stereo-

chemistry of the side chain of **1** was deduced to be the same as that of perulactone B by comparison of the chemical shifts and the coupling constants of H-22 and H-28 (Tables 1 and 2). In addition, the NOE correlations of H-28a with H-24 and H-28b, of H-24 with H-22 and H-25, and of Me-27 with H-25 were observed in the ROESY spectrum (Fig. 3), which also further confirmed the configuration of the side chain. On the basis of all the above evidence, the structure of **1** was established as (20*S*,22*R*,24*S*,25*R*)-5β,6β-epoxy-4β,14α,17β,20,22-pentahydroxy-1-oxoergosta-2-en-26,28-olide.

Perulactone F (**2**) had the molecular formula $C_{28}H_{38}O_9$, as established from HRESI-MS (*m/z* 541.2413 $[M+Na]^+$, calcd. for 541.2413). The ^1H and ^{13}C NMR (Table 1) spectra of **2** were nearly identical to those of **1**, except for the signals concerning ring A. In the ^1H NMR spectrum (Table 1), an AB system at δ_H 6.89 (1H, d, J = 10.3 Hz, H-3) and 6.84 (1H, d, J = 10.3 Hz, H-2), combined with the signals at δ_C 202.1, 141.6, 139.3, and 193.9 in the ^{13}C NMR spectrum assigned to C-1, C-2, C-3, and C-4, suggested the presence of a Δ^2-1,4-diketone system in ring A [17–19]. This substitution pattern was further confirmed by the following HMBC correlations: H-2 with C-4 and C-10; H-3 with C-1 and C-5; and H-19 with C-1, C-5, C-9 and C-10. The remaining spectroscopic features of **2** were also similar to those of perulactone B obtained in $CDCl_3$ (Tables 1 and 2). Therefore, the structure of perulactone F was considered to be (20*S*,22*R*,24*S*,25*R*)-5β,6β-epoxy-14α,17β,20,22-tetrahydroxy-1,4-dioxoergosta-2-en-26,28-olide.

Perulactone G (**3**) gave the same molecular formula of $C_{28}H_{40}O_9$ as **1** on the basis of positive HRESI-MS and NMR data. A comparison of the NMR data of these two compounds indicated that they were quite similar, with the main differences being in the A/B rings. The ^1H NMR spectrum of **3** (Table 1) showed two oxymethine signals as a doublet at δ_H 4.21 (J = 4.0 Hz, H-4) and a double doublet at δ_H 4.58 (J = 10.4, 4.8 Hz, H-6), which together with the chemical shifts of C-4 (δ_C 49.2, CH), C-5 (δ_C 69.6, C), and C-6 (δ_C 64.9, CH), suggested the presence of a 4,5-epoxy-6-hydroxy moiety in **3**. The relative configuration of **3** was determined on the basis of the coupling constants and ROESY correlations. The coupling constants observed between H-6/H-7*ax* (J = 10.4 Hz), and H-6/H-7*eq* (J = 4.8 Hz), and the ROESY correlation of H-6 with Me-19 indicated that H-6 was axial and that the hydroxyl group was therefore α-oriented [20]. In addition, a weak cross-peak between H-4 and H-7α was observed in the ROESY spectrum, which supported a β-orientation of the 4,5-epoxy group. The other relative stereochemistry of **3** was in good agreement with that of **1** due to the similarities between the observed ROESY correlations and those of **1**. Thus, the structure of **3** was determined to be (20*S*,22*R*, 24*S*,25*R*)-4β,5β-epoxy-6α,14α,17β,20,22-pentahydroxy-1-oxoergosta-2-en-26,28- olide.



1

Fig. 2. ^1H,^1H-COSY(—) and key HMBC(→) correlations of **1**.

The molecular formula of perulactone H (**4**) was determined to be $C_{28}H_{40}O_7$ by the $[M+Na]^+$ ion peak at *m/z* 511.2672 (calcd. for 511.2671) in HRESI-MS. The IR, UV and NMR spectra of **4** showed features very similar to those of perulactone B [3]. A detailed comparison of the ^{13}C NMR data of these two compounds indicated that they have the same planar structure. The key differences were the signals for carbons around C-17, which indicated that **4** should be the 17-epimer of perulactone B. Pyridine, as a useful solvent, is known to form weak hydrogen bonds with hydroxyl groups and can influence the chemical shifts of neighboring protons [21]. The observed chemical shifts in different solvents ($\Delta = \delta_{CDCl3} - \delta_{C5D5N}$) are useful in determining the position and orientation of hydroxyl groups [21]. The orientation of OH-17 could be deduced by the pyridine-induced shifts of Me-18, Me-21 and H-22 [11]. The observed differences in the ^1H NMR chemical shifts of **4** (Table 2) for Me-18, Me-21 and H-22 were −0.50, −0.44 and −0.37, respectively. Compared with the results for perulactone B, the Me-18 showed a significant shift due to 20β-OH being very close to Me-18, which suggested that OH-17 has an α-orientation in **4**. However, in the ^{13}C NMR spectrum of **4**, the chemical shifts of the carbon atoms in rings C/D are in good agreement with those reported for withanolides with 14α-OH/17α-OH groups, which also suggested an α-orientation for 17-OH [3,16]. Thus, the structure of **4** was elucidated as (20*S*,22*R*,24*S*,25*R*)-14α,17α,20,22- tetrahydroxy-1-oxoergosta-2,5-dien-26,28-olide.

Based on the positive HRESI-MS data, the molecular formula of withaperuvin I (**5**) was determined to be $C_{29}H_{42}O_9$. The IR spectrum showed absorptions for hydroxyl groups at 3424 cm^{-1} and for an α,β-unsaturated ketone at 1692 cm^{-1}. Five methyl signals at δ_H 1.46 (3H, s, Me-18), 1.66 (3H, s, Me-19), 1.84 (3H, s, Me-21), 2.01 (3H, s, Me-27), and 2.97 (3H, s, 5-OCH_3), one hydroxymethylene group at δ_H 4.65 (1H, d, J = 14.4 Hz, H-28a) and 4.36 (1H, d, J = 14.4 Hz, H-28b), two oxymethine protons at δ_H 4.27 (1H, brs, H-6) and 5.39 (1H, dd, J = 13.2, 2.4 Hz, H-22), and two olefinic protons at δ_H 6.01 (1H, dd, J = 10.0, 2.4 Hz, H-2) and 6.51 (1H, dd, J = 10.0, 3.6 Hz, H-3) were displayed in the ^1H NMR spectrum (Table 3). The ^{13}C NMR spectrum of **5** (Table 3) revealed five methyl, eight methylene, six methine, and ten quaternary carbons. The similarities between the NMR data of **5** and those of phyperunolide B (**12**) [8] indicated that **5** was a 28-hydroxywithanolide. The only exception is that the hydroxyl group in **12** was replaced by a methoxyl group in **5**. The HMBC correlations (Fig. 4) between the methoxyl group at δ_H 2.97 and C-5 (δ_C 82.8, C) indicated the methoxyl group was located at C-5. The correlations of 5-OCH_3 with H-4α and H-6α observed in the ROESY spectrum (Fig. 5) suggested the α-orientation of 5-OMe. The configuration of **5** at C-22 was determined as *R* on the basis of the H-22 coupling constants (J = 13.2, 2.4 Hz), which indicated axial–axial and axial–equatorial relationships of H-22 with the C-23 protons [22]. The rest of the configuration of **5** was determined to be the same as that of **12** by comparison of the NMR spectral data [8]. Consequently, compound **5** was established as (20*S*,22*R*)-6β,14α,17β,20,28-pentahydroxy-5α-methoxy-1-oxowitha-2,24-dien-26,22-olide.

Withaperuvin J (**6**) possessed a molecular formula of $C_{28}H_{38}O_7$ on the basis of the HRESI-MS with $[M+Na]^+$ at *m/z* 509.2512 (calcd. for C28H38O7Na, 509.2515). Analysis of its ^{13}C NMR spectral data (Table 3) revealed that the structure of **6** was similar to that of **5**. The key differences between these two compounds were the appearance of two olefinic carbon signals at δ_C 135.0 (C, C-5) and 125.3 (CH, C-6) in **6** instead of those from the 5-methoxy-6-hydroxy unit in **5**. HMBC correlations of the olefinic proton signal at δ_H 5.60 with C-4 (δ_C 33.5, CH_2), C-8 (δ_C 37.3, CH), and C-10 (δ_C 50.7, C), and of H-3 (δ_H 6.77, ddd, J = 10.0, 4.8, 2.4 Hz), H-4α (δ_H 2.83, dd, J = 21.2, 4.8 Hz), and Me-19 (δ_H 1.22) with C-5 suggested the presence of a double bond between C-5 and C-6 in **6**. The relative configurations of **6** were established to be identical

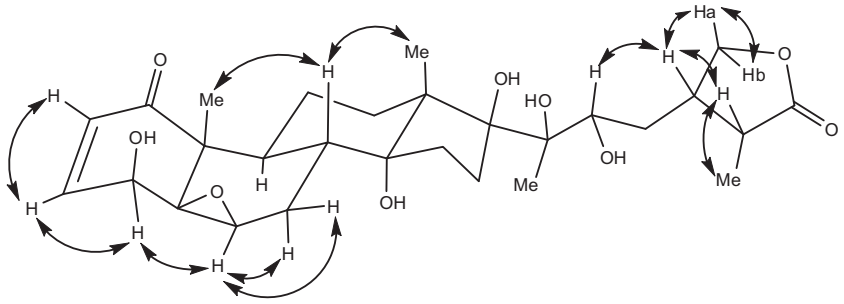


Fig. 3. Selected ROESY (↷) correlations for **1**.

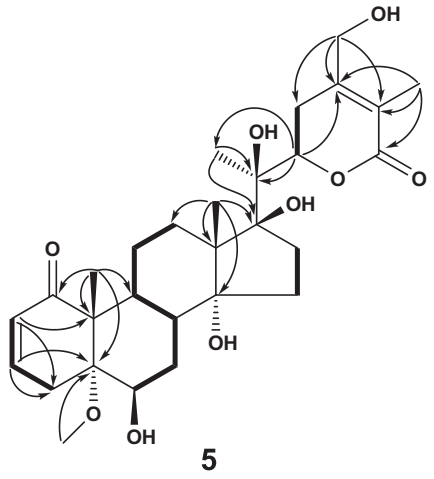


Fig. 4. ^{1}H,^{1}H-COSY(—) and key HMBC(→) correlations of **5**.

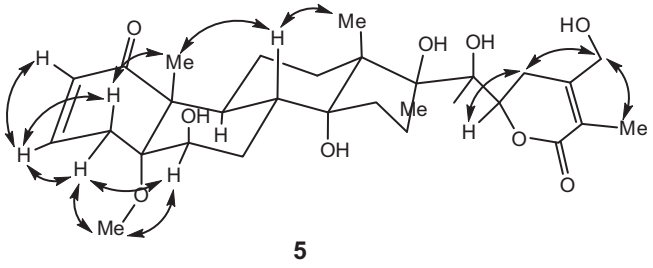


Fig. 5. Selected ROESY (↷) correlations for **5**.

to those of **5** by their similar ROESY correlations. Thus, compound **6** was determined as (20*S*,22*R*)-14α,17β,20,28-tetrahydroxy-1-oxowitha-2,5,24-trien-26,22-olide.

Withaperuvin K (**7**) was determined to have the molecular formula $C_{29}H_{42}O_{10}$ based on the positive HRESI-MS. The ^{1}H and ^{13}C NMR spectroscopic data of **7** (Table 3) were very close to those of **5**; the main differences between them were the functional groups of rings A and B. Because of the near overlap of two oxygenated methane signals in the ^{1}H NMR spectrum in C_5D_5N, the 1D and 2D NMR spectra of **7** in $CDCl_3$ were also recorded. The ^{1}H and ^{13}C NMR spectra of **7** in $CDCl_3$ showed the presence of a methoxyl group [δ_H 3.25 (3H, s) and δ_C 56.9], a characteristic methylene [δ_H 2.73 (1H, dd, J = 15.4, 7.8 Hz, H-2a) and 2.61 (1H, dd, J = 15.4, 3.8 Hz, H-2b)], two oxymethines [δ_H 3.56 (1H, m, H-3), 3.36 (1H, d, J = 2.5 Hz, H-4) and δ_C 78.7 (CH, C-3), 74.7(CH, C-4)], an epoxy group [δ_H 3.21 (1H, s) and δ_C 65.0 (qC, C-5), 59.7 (CH, C-6)] and a non-conjugated carbonyl group [δ_C 211.3 (qC, C-1)]. These data and the ^{1}H,^{1}H-COSY spectra of **7** indicated that **7** has a characteristic 4β-hydroxy-3β-methoxy-5β,6β-epoxy-1-one structural unit in rings A and B [23,24]. The methoxyl group was located at C-3 because the HMBC correlation between the methoxyl signal at δ_H 3.25 and C-3 was observed. The orientation of H-3, H-4 and H-6 was deduced from the ^{1}H NMR coupling constants [24] and the observed ROESY correlations. The $J_{2\alpha,3}$, $J_{2\beta,3}$ and $J_{3,4}$ coupling constants (3.8, 7.8, and 2.5 Hz, respectively) in $CDCl_3$, together with the ROESY correlations of H-3 with H-2α, H-4 and 3-OMe, of 3-OMe with H-2β, and of H-4 with H-6 indicated that the H-3, H-4 and H-6 should be α-oriented. In addition, a pyridine-induced shift of Me-19 (Δ −0.56) also suggested that both OH-4 and Me-19 have a β-orientation [24]. Therefore, the structure of withaperuvin K was considered to be (20*S*,22*R*)-5β,6β-epoxy-4β,14α,17β,20,28-pentahydroxy-3β-methoxy-1-oxowitha-24-en-26,22-olide.

Withaperuvin L (**8**) was isolated as an amorphous solid. The molecular formula, $C_{28}H_{38}O_8$, was deduced from the HRESI-MS (*m/z* 525.2470 [M + Na]$^+$, calcd. for 525.2464). A detailed comparison of the ^{1}H and ^{13}C NMR data of **8** (Tables 4 and 5) with those of **5** showed that they were closely comparable in structure; the differences between these two compounds appeared in ring B and at C-28. In the NMR spectra of **8**, the absence of a two doublet adscript to 28-CH_2OH, along with HMBC correlations of one more methyl group at δ_H 1.93 with C-23, C-24, and C-25, indicated no hydroxyl group at C-28. In the ^{1}H and ^{13}C NMR spectra (in $CDCl_3$), two oxymethine proton signals at δ_H 3.21 (1H, br s, H-6) and 4.18 (1H, d, J = 8.9 Hz, H-7) associated with three oxygenated carbon signals at δ_C 65.7 (C, C-5), 67.6 (CH, C-6) and 67.8 (CH, C-7) revealed a 5,6-epoxy-7-hydroxy unit in ring B [25], which was confirmed by the HMBC correlations of H-6 with C-4, C-7 and C-8, and of H-7 with C-6, C-8 and C-14. Additionally, acetylation of **8** yielded the monoacetylated derivative **8a**. In the ^{1}H NMR spectrum of **8a**, the downfield shift of H-7 from δ_H 4.18 to 5.37 also indicated the location of a hydroxyl group at C-7. The relative stereochemistry in ring B was elucidated by analysis of the coupling constant values and the ROESY spectrum. Because of the near overlap of two proton signals (H-8 and H-9,) in $CDCl_3$, the NMR data of **8** in C_5D_5N were also recorded. The ROESY correlations (in C_5D_5N) of H-7 with H-6 and H-9, and of H-6 with H-4α, suggested that the 5,6-epoxide and 7-OH were both β-oriented. Moreover, a larger coupling of H-7 with H-8 implied a pseudoaxial proton was located at C-7; a broad singlet signal of H-6 further confirmed the α-orientations of both H-6 and H-7. Thus, the structure of **8** was established as (20*S*,22*R*)-5β,6β-epoxy-7β,14α,17β,20-tetrahydroxy-1-oxowitha-2,24-dien-26,22- olide.

Withaperuvin M (**9**) was obtained as a pale-yellow solid with a molecular formula of $C_{28}H_{36}O_7$ as determined by the HREI-MS (*m/z* 484.2455 [M]$^+$, calcd. for 484.2461). Comparison of the NMR data of **9** and **8** (Tables 4 and 5) indicated they have identical C/D rings and side chains; the difference was the substitution patterns of

rings A and B. In the ^{1}H NMR spectrum (Table 4) of **9**, an A/B system at δ_H 6.70 (1H, d, J = 10.3 Hz, H-3) and 6.64 (1H, d, J = 10.3 Hz, H-2), an olefinic proton at δ_H 6.89 (1H, dd, J = 5.6, 2.4 Hz, H-6), together with the carbon signals at δ_C 202.0 (C, C-1), 139.1 (CH, C-2), 140.3 (CH, C-3), 187.9 (C, C-4), 138.8 (C, C-5) and 139.1 (CH, C-6) in the ^{13}C NMR spectrum (Table 5), suggested the presence of a 1,4-diketone-2,5-diene system in the molecule [18,26]. This substitution pattern was also confirmed by the long-range correlations of H-2 with C-4, C-5, and C-10, of H-3 with C-1 and C-5, and of H-6 with C-4, C-8 and C-10 in the HMBC experiment. Given this evidence, the structure of **9** was assigned as (20*S*,22*R*)-14α,17β,20-trihydroxy-1,4-dioxowitha-2,5,24-trien-26,22-olide.

Withaperuvin N (**10**) gave a molecular formula of $C_{28}H_{40}O_9$ by HRESI-MS, which indicated nine degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **10** (Tables 4 and 5) were similar to those of **8**; the main differences are that **10** possesses the 5,6,7-triol unit in ring B rather than the 5β,6β-epoxy-7β-hydroxyl groups of **8**. These differences were evident from the oxygenated carbon signals at δ_C 78.7, 79.4 and 68.3, which were assigned to C-5, C-6 and C-7; the downfield shifts of the former two carbons suggested the presence of a typical 5α,6β-diol group in the molecule. Moreover, acetylation of **10** afforded the diacetylated derivative **10a**, and the downfield shifts of the H-6 from δ_H 4.23 to 5.90 and of the H-7 from δ_H 5.20 to 6.58 indicated that two hydroxyl groups are located at the C-6 and C-7 positions. The large coupling observed for H-7 with H-8 (J = 10.4 Hz) and the small coupling for H-7 with H-6 (J = 3.2 Hz) revealed that the H-7 was in an axial position, whereas the H-6 was in an equatorial position, which suggested the β-orientation of both 6-OH and 7-OH groups [27]. These assignments were also confirmed by the ROESY correlations of H-7 with H-9 and H-6 with H-4α. Therefore, compound **10** was identified as (20*S*,22*R*)-5α,6β,7β,14α,17β,20-hexahydroxy-1-oxowitha-2,24-dien-26,22-olide.

In addition, 3- and 5-methoxy derivatives **5**, **7**, **13** and **16** were assumed to be artifacts resulting from the treatment with MeOH during the process of extraction and purification. These compounds were probably generated by Michael-type additions and epoxide ring-opening reactions with solvents [24,28].

Acknowledgments

The project was supported by grants from the Fund of State Key Laboratory of Phytochemistry and Plant Resources in West China (P2008-ZZ14), and the Natural Science Foundation of Yunnan Province (No. 2007C091M).

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