Antileishmanial Physalins from *Physalis minima*

by **M. Iqbal Choudhary***, **Sammer Yousaf**, **Shakil Ahmed**, **Samreen**, **Kauser Yasmeen**, and **Atta-ur-Rahman***

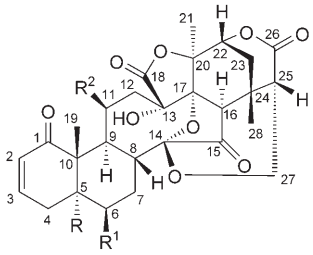
H.E.J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi-75270, Pakistan
(phone: +92-21-9243224, 9243211; fax: +92-21-9243190-91; e-mail: hej@cyber.net.pk)

Three new physalins (**1**–**3**) and a new withanolide **7** have been isolated from the whole plant of *Physalis minima*, along with three known physalins: physalin H (**4**), isophysalin B (**5**), and 5*β*,6*β*-epoxyphysalin B (**6**). Their structures were deduced on the basis of in-depth spectroscopic analyses. Compounds **1**–**6** showed significant *in vitro* leishmanicidal activities (0.92–19.4 μg/ml) against promastigotes of *Leishmania major*.

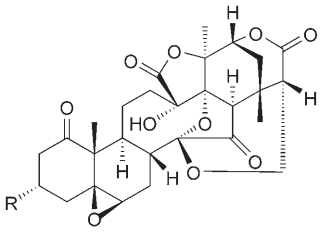
Introduction. – Physalins are the steroidal lactone constituents of *Physalis* and other closely related genera, belonging to the family Solanaceae [1]. Normal withanolides like withaferin A are C_{28} steroidal compounds possessing a relatively highly oxidized ergostane-type skeleton characterized by a six-membered lactone ring in the side chain [2][3]. The physalins are biogenetically related to the withanolides from which they are formally derived by *a*) oxidative bond cleavage between C(13) and C(14) to yield a nine-membered ring, *b*) formation of a new six-membered carbocycle between C(16) and C(24), and *c*) oxidation of the Me group at C(13) to a COOH group, which results in 18,20-lactonization [3]. Physalins are commonly named as 16,24-cyclo-13,14-seco-steroids [1].

The plants of the genus *Physalis* possess a number of interesting biological properties including anti-inflammatory [4], quinone reductase induction [5], immunomodulatory [6][7], antimycobacterial [8][9], and hypoglycemic activities [10]. The genus *Physalis* is also used in Guatemala for the treatment of gonorrhea [11], and *P. minima* (LINN. Var. *indica*) is a herb widely used in folk medicines; the roots are used as a vermifuge and febrifuge, and for diabetes [12]. The variations in the C_{28} steroidal skeleton of the physalins and the great biological activities of this class of compounds prompted us to extend our studies on *P. minima*, which resulted in the isolation of three new (**1**–**3**) and three known (**4**–**6**) physalins, along with a new withanolide (**7**), some of which showed significant leishmanicidal activities.

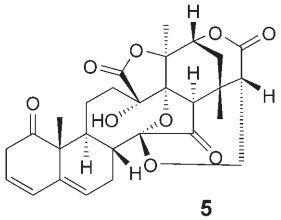
Leishmaniasis is caused by protozoa parasites of the genus *Leishmania*, a biologically diverse group of flagellate parasites of the Tryanosomatidae family, which can be differentiated by genetic, biochemical, and immunological studies. Leishmaniasis typically occurs in the Old World around the Mediterranean sea, in East and West Africa, Afghanistan, Pakistan, India, Nepal, Bangladesh, and in China. In the New World, this disease is found in the southern parts of the United States to the northern parts of Argentina and Paraguay. Both domestic and wild animals are main reservoirs of *Leishmania* parasites, while the female flying insects of the genera



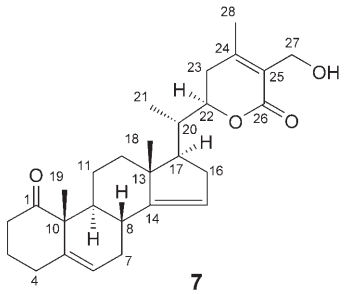
1 R = R^1 = R^2 = OH
3 R = OH, R^1 = R^2 = H
4 R = Cl, R^1 = OH, R^2 = H



2 R = MeO
6 R = H, $\Delta^{2,3}$



5



7

Phlebotomus and *Lutzomya* are the vectors of leishmaniasis. The secondary metabolites of plants, alkaloids, quinones, and terpenes, have been used to cure protozoan parasitic diseases. The use of quinine and emetine are best examples for the treatment of parasite diseases such as malaria and amoebiasis [13].

Some natural products have also shown leishmanicidal activities. Diospyrin, isolated from *Diospyros mantana,* is active against *L. donovani* [14]; berberine is effective against cutaneous leishmaniasis in rats; and harmaline, which was isolated from *Peganum harmala*, shows antiprotozoal action [15][16]. As the parasitic diseases spread into new areas of the world, and since causative agents develop resistance against the prevailing therapies, the search for novel plant-derived agents effective against parasites continues with vigor.

Results and Discussion. – Compound **1** was obtained as a colorless, amorphous powder. HR-EI-MS showed the M^+ signal at *m/z* 560.5457, in agreement with the formula $C_{28}H_{32}O_{12}$ (calc. 560.5465). The IR spectrum showed a strong absorption at 3432 cm^{-1}, indicating the presence of OH groups. The absorption band at 1778 cm^{-1} is due to a five-membered lactone ring characteristic of all physalins [1][12][17]. Furthermore, the IR absorption at 1670 cm^{-1} was assigned to an α,β-unsaturated ketone [18], in agreement with a UV absorption maximum at 226 nm [12].

The ^{1}H-NMR spectroscopic data (*Table 1*) of compound **1** showed three characteristic Me *singlets* at δ (H) 1.25, 1.35, and 1.99, which were assigned to C(28), C(19), and C(21), respectively [1][12][19]. The presence of an ether bridge between C(14) and C(27) was inferred from the appearance of a pair of characteristic signals for CH_2(27), δ(H) 3.75 (*d*, *J*(27,27) = 13.5) and 4.53 (*dd*, *J*(27,27) = 13.5, *J*(27,25) = 4.4) [19]. The

Table 1. *^{1}H-NMR Data of the New Compounds* **1**–**3** *and* **7**. At 400 MHz; δ in ppm, J in Hz; assignments based on COSY and HMQC experiments.

Position	**1**[a])	**2**[b])	**3**[b])	**7**[b])
2	5.62 (*dd*, $J(2,3)=10.2$, $J(2,4\beta)=2.6$)	2.56–2.78 (*m*) 2.82 (*dd*, $J(2\alpha,2\beta)=13.7$, $J(2\alpha,3)=2.8$)	5.98 (*dd*, $J(2,3)=10.1$, $J(2,4\beta)=2.2$)	2.25–2.45 (*m*)
3	6.72 (*ddd*, $J(3,2)=10.2$, $J(3,4a)=4.8$, $J(3,4\beta)=2.8$)	3.68–3.88 (*m*, $W_{1/2}=9.6$)	6.84 (*ddd*, $J(3,2)=10.0$, $J(3,4\alpha)=5.2$, $J(3,4\beta)=2.1$)	1.95–2.25 (*m*)
4	3.55 (*dt*, $J(4\alpha,4\beta)=21.5$) 2.26–2.42 (*m*)	2.13–2.46 (*m*)	2.05–2.25 (*m*)	1.62–1.75 (*m*)
6	4.10 (br. *s*, $W_{1/2}=4.6$)	3.22 (*d*, $J(6\alpha,7\beta)=2.2$)	2.82 (*dd*, $J(6,7\beta)=13.8$, $J(6,7\beta)=2.9$)	5.89 (br. *s*)
7	2.86–2.89 (*m*)	2.13–2.35 (*m*)	1.15–1.45 (*m*)	2.11–2.32 (*m*)
8	2.32 (*d*, $J(8,9)=8.3$)	1.50–1.78 (*m*)	2.02–2.22 (*m*)	1.52–1.73 (*m*)
9	3.12–3.56 (*m*)	1.88–1.99 (*m*)	3.12–3.56 (*m*)	1.65–1.91 (*m*)
11	4.01 (br. *s*, $W_{1/2}=4.5$)	1.55–1.68 (*m*)	2.49–2.69 (*m*)	–
12	2.93–3.01 (*m*)	1.10–1.25 (*m*)	2.19–2.24 (*m*)	1.75–1.90 (*m*)
15	–	–	–	5.58–5.82 (*m*)
16	2.67–2.85 (*m*)	2.01–2.25 (*m*)	3.21 (*s*)	1.65–1.90 (*m*)
17	–	–	–	0.07–1.02 (*m*)
18	–	–	–	1.04 (*s*)
19	1.35 (*s*)	1.23 (*s*)	1.28 (*s*)	1.10 (*s*)
20	–	–	–	1.65–1.95 (*m*)
21	1.99 (*s*)	1.96 (*s*)	1.92 (*s*)	1.25 (*d*, $J(21,20\alpha)=3.6$)
22	4.55 (*t*, $J(22,23)=2.9$)	4.53 (*t*, $J(22,23)=5.4$)	4.55 (*d*, $J(22,23)=3.6$)	4.44 (*dd*, $J(22\alpha,23\alpha)=13.2$, $J(22\alpha,23\beta)=5.1$)
23	2.32–2.50 (*m*)	1.88–1.99 (*m*)	2.10 (*s*)	2.10–2.32 (*m*)
25	2.48–2.63 (*m*)	2.01–2.45 (*m*)	2.01–2.45 (*m*)	–
27	3.75 (*d*, $J(27,27)=13.5$) 4.53 (*dd*, $J(27,27)=13.5$, $J(27,25)=4.4$)	3.73 (*d*, $J=13.5$) 4.48 (*dd*, $J(27,27)=13.5$, $J(27,25)=4.5$)	3.72 (*d*, $J(27,27)=13.6$) 4.48 (*dd*, $J(27,27)=13.4$, $J(27,25)=4.5$)	4.36 (*d*, $J_{AB}=12.5$) 4.38 (*d*, $J_{AB}=12.5$)
28	1.25 (*s*)	1.02 (*s*)	1.23 (*s*)	1.85 (*s*)
3-MeO	–	3.62 (*s*)	–	–

[a]) In CD_3OD. [b]) In $CDCl_3$.

coupling of $CH_2(27)$ with H–C(25) at δ(H) 2.58, confirmed by a COSY-45° experiment, further supported the presence of an OCH_2 bridge. Two mutually coupled olefinic signals resonated at δ(H) 5.62 (*dd*, $J(2,3)=10.2$, $J(2,4\beta)=2.6$) and at 6.72 (*ddd*, $J(3,2)=10.2$, $J(3,4\alpha)=4.8$, $J(3,4\beta)=2.8$), indicating a 2-ene-1-one system in ring *A* [17]. A broad *singlet* at δ(H) 4.10 (half-width 4.6 Hz) was assigned to H–C(6) geminal to an OH group. Another downfield one-proton signal at δ(H) 4.01 (half-width 4.5 Hz) was assigned to H–C(11) geminal to an OH group. H–C(11) also showed HMBC correlations with C(12) (δ(C) 32.2) and C(13) (80.4) (*Fig. 1*). This H–C(11) was

deduced to be equatorial, based on the lower value of its half-width (4.6 Hz) [20]. In the NOESY spectrum, the interaction between H–C(11) at δ(H) 4.01 and H_α–C(9) at 3.22, further supported an equatorial orientation of H–C(11).

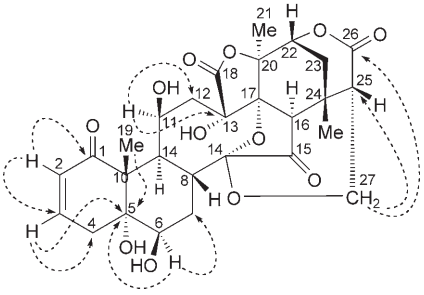


Fig. 1. *Key HMBC interactions for Compound* **1**

The ^{13}C-NMR data of compound **1** (*Table 2*) showed signals for 28 C-atoms. A downfield quaternary-carbon signal at δ (C) 78.8 was assigned to the oxygenated C(5)-atom. This assignment was further supported by the HMBC interactions between H–C(6) at δ(H) 4.10 and Me(19) at δ(H) 1.35 with C(5) at δ(C) 78.8 (*Fig. 1*). The configuration at C(5) was deduced by comparing the ^{13}C-NMR data with literature values [21]. The basic skeleton of physalins has previously been determined by single-crystal diffraction analysis [22][23]. From the aforementioned data, the structure of compound **1** was deduced as 16,24-cyclo-13,14-secoergost-2-ene-18,26-dioic acid 14 : 17,14 : 27-diepoxy-5α,6β,11β,13,20,22-hexahydroxy-1,15-dioxo-γ-lactone δ-lactone[1]).

Compound **2** was obtained as a yellowish gummy material. HR-EI-MS showed the molecular ion at *m/z* 558.5740 (M^+), in agreement with the formula $C_{29}H_{34}O_{11}$ (calc. 558.5736). The IR spectrum was largely similar to that of compound **1**, but no absorption band was observed for an α,β-unsaturated ketone. A UV absorption at 201 nm further supported the lack of an α,β-unsaturated ketone [18].

The ^{1}H-NMR spectrum of compound **2** (*Table 1*) showed a number of signals characteristic of the physalin skeleton. The signal at δ(H) 3.22 (J(6α,7β) = 2.2) was assigned to H–C(6), geminal to an oxirane ring. The low J value and comparison of the chemical shift with a known physalin (5β,6β-epoxyphysalin B) suggested α-orientation of H–C(6) [3]. A downfield three-proton *singlet* at δ(H) 3.26 was assigned to the 3-MeO group. A *multiplet* at δ(H) 3.68–3.88 (half-width 9.6 Hz) was assigned to H–C(3), as further supported by the ^{1}H,^{1}H connectivities between H–C(3) and CH_2(2) (δ(H) 2.56–2.78, 2.82) and CH_2(4) (2.13–2.46), and by the HMBC interactions between H–C(3) proton (δ(H) 3.68–3.88) and C(1) (δ(C) 207.6), C(2) (42.3), and C(4) (36.6) (*Fig. 2*). The low half-width of the H–C(3) signal indicated its pseudoequatorial orientation [24]. On the basis of the presented spectral data, compound **2** was identified as 16,24-cyclo-13,14-secoergosta-18,26-dioic acid 5,6 : 14 : 17,14 : 27-triepoxy-13,20,22-trihydroxy-3α-methoxy-1,15-dioxo-γ-lactone δ-lactone[1]).

---

[1]) For systamtic names, see *Exper. Part.*

Table 2. *^{13}C-NMR Data of Compounds* **1**–**3** *and* **7**. At 100 MHz; δ in ppm, assignments based on HMQC and HMBC experiments.

Position	**1**[a])	**2**[b])	**3**[b])	**7**[b])
1	203.5	207.6	207.4	199.8
2	127.8	42.3	127.7	34.0
3	143.3	73.4	146.3	29.8
4	37.2	36.6	36.6	41.0
5	78.8	63.8	76.4	142.0
6	73.9	63.4	42.2	126.7
7	31.1	29.0	29.6	38.2
8	38.6	36.7	37.3	41.0
9	30.9	33.7	34.2	39.2
10	54.6	53.8	50.0	51.4
11	68.0	23.3	24.9	24.0
12	32.2	25.6	23.5	22.8
13	80.4	80.0	80.1	49.6
14	107.3	107.0	107.0	154.1
15	208.3	213.2	210.2	128.9
16	56.0	56.0	56.2	40.0
17	79.8	81.0	80.0	52.0
18	172.1	172.3	170.1	15.4
19	21.2	21.5	21.5	16.7
20	80.9	81.0	80.3	30.1
21	26.5	26.5	26.5	13.9
22	77.0	76.8	76.8	78.9
23	32.9	33.0	33.0	28.2
24	31.1	31.1	31.0	154.9
25	50.9	50.8	50.8	126.8
26	166.9	166.5	166.9	163.0
27	60.7	60.7	60.6	58.2
28	14.5	15.1	19.2	15.6
3-MeO	–	56.2	–	–

[a]) In CD_3OD. [b]) In $CDCl_3$.

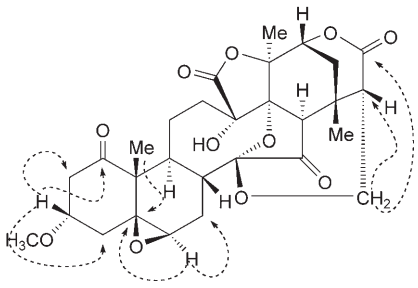


Fig. 2. *Key HMBC Interactions for Compound* **2**

Compound **3** was obtained as a colorless powder. HR-EI-MS showed the M^+ signal at *m/z* 528.5465, in agreement with the formula $C_{28}H_{32}O_{10}$ (calc. 528.5477). The IR and UV spectra of **3** were distinctly similar to those of **1**, indicating the presence of OH groups (3434 cm^{-1}) and a five-membered lactone (1778 cm^{-1}).

The ^{1}H- and ^{13}C-NMR data of **3** (*Tables 1* and *2*) were very similar to those of **1**. In the ^{13}C-NMR spectrum, a downfield quaternary signal at δ (C) 76.4 was assigned to the OH-substituted C(5)-atom. This assignment was further supported by HMBC interactions between C(5) and CH_2(4) (δ(H) 2.05–2.15) and C(19) (δ(H) 1.28) (*Fig. 3*). On the basis of all spectroscopic data, the structure of **3** was deduced as 16,24-cyclo-13,14-secoergost-2-ene-18,26-dioic acid 14:17,14:27-diepoxy-5α,13,20,22-tetrahydroxy-1,15-dioxo-γ-lactone δ-lactone[1]).

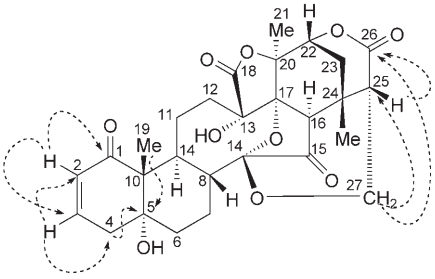


Fig. 3. *Key HMBC Interactions for Compound* **3**

The new withanolide **7** was obtained as a yellowish gum. Its IR spectrum showed absorptions at 3411, 1715, and 1657 cm^{-1}, assigned to OH, α,β-unsaturated δ-lactone, and C=C functionalities, respectively [25]. Compound **7** showed a UV absorption band at 201 nm characteristic of a saturated hexanone [18]. HR-EI-MS showed the M^+ signal at *m/z* 438.5978, corresponding to the formula $C_{28}H_{38}O_4$ (calc. 438.5989).

The ^{1}H-NMR spectrum of **7** (*Table 1*) showed three characteristic *singlets* at δ(H) 1.04, 1.10, and 1.85 for Me(18), Me(19), and Me(28), respectively, and a *doublet* at 1.25 (J(21,20α) = 3.6, 3 H) was due to Me(21). A downfield signal at δ(H) 4.44 (*dd*; J(22α,23α) = 13.2, J(22α,23β) = 5.1) was assigned to H–C(22) of the δ-lactone ring. Two-proton *AB doublets* at δ(H) 4.36 and 4.38 (J_{AB} = 12.5) were due to the hydroxylated CH_2(27) group. All above observations convincingly supported a tetracyclic steroidal skeleton with a lactone substituent [2][3] [25–27]. A downfield broad *singlet* at δ(H) 5.89 was assigned to the vinylic H–C(6) proton [24], and another downfield *multiplet* at δ(H) 5.58–5.82 was due to the vinylic H–C(15) [25].

The ^{13}C-NMR spectrum of **7** (*Table 2*) showed signals for 28 C-atoms. Two quaternary ^{13}C signals at δ(C) 142.0 and 154.1 were assigned to C(5) and C(14), respectively, whereas two downfield signals at δ(C) 126.7 and 128.9 were due to the olefinic C(6) and C(15) atoms. The positions of the C=C bonds were further inferred by HMBC experiments (*Fig. 4*). From these data, **7** was identified as 27-hydroxy-1-oxowitha-5,14,24-trienolide.

Comparison of the spectroscopic data of compounds **4**–**6** with those reported led to their identification as physalin H [28], isophysalin B [17], and 5β,6β-epoxyphysalin B, respectively [3][29]. These compounds had first been reported from *P. angulata*, *P. alkekengi*, and *P. minima*, respectively.

Compounds **1**–**7** were screened for their antileishmanial activities in an *in vitro* assay. Compounds **1**, and **3**–**6** were found to have significant antileishmanial activities. Amphotericin (IC_{50} = 0.19 μg/ml) was used as a standard drug. Compound **1**,

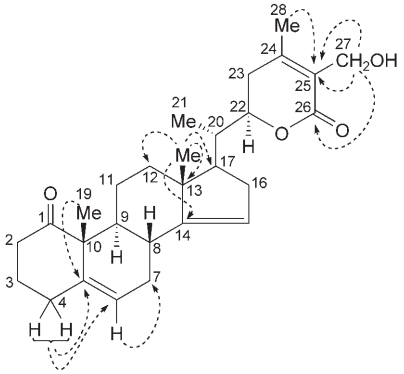


Fig. 4. *Key HMBC Interactions for Compound* **7**

containing OH groups at C(5), C(6), and C(11), was found to be the most active in this series, with an IC_{50} value of 0.92 μg/ml (*Table 3*).

Table 3. In vitro *Antileishmanial Activities of Compounds* **1**–**7**

Compounds	IC_{50} [μg/ml][a])
1	0.92 ± 0.001
2	19.4 ± 0.18
3	5.00 ± 0.01
4	3.39 ± 0.005
5	7.05 ± 0.05
6	3.39 ± 0.005
7	38.9 ± 0.105
Amphotericin[b])	0.12 ± 0.105

[a]) Standard deviations for seven assays. [b]) Positive control.

S. Y., acknowledges the *Higher Education Commission*, Islamabad, Pakistan, for financial support through the 'Merit Scholarship Scheme for Ph.D. Studies in Science and Technology'.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 70–230 mesh). Optical rotations: *Polartronic-D* polarimeter (*Schmidt & Haensch*). UV Spectra: *Hitachi U-3200* spectrophotometer; λ_{max} in nm ($\log \varepsilon$). IR Spectra: *Jasco FT-IR-8900* spectrophotometer; in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker Avance-400* and *-500* spectrometers at 400/100 and 500/125 MHz, resp., in $CDCl_3$ or CD_3OD; δ in ppm, J in Hz. FAB-MS (pos. mode, with Xe) and HR-EI-MS (70 EV, ion-source temp. 250°): *Jeol JMS-600* and *HX-110* mass spectrometers with the data system *DA-5000*; in *m/z* (rel. %).

Plant Material. Fresh plants (80.6 kg) of *P. minima* (Linn. Var. *indica*), were collected from Karachi (Pakistan), and identified by a plant taxonomists, Botany Department, University of Karachi. A voucher specimen (G. H. 68261) was deposited at the herbarium of the University of Karachi.

Extraction and Isolation. The air-dried plants (25.8 kg) were extracted with MeOH (120 l) for 21 d. After evaporation of the solvent, a residue (2.5 kg) was obtained, which was dissolved in distilled H_2O (5 l), and extracted (defatted) with petroleum ether (10 l). The resulting aq. extract was further extracted with CH_2Cl_2 (35 l) and then BuOH (5 l). The CH_2Cl_2 extract was concentrated to a gum (350.5 g), and subjected to CC (SiO_2; CH_2Cl_2/MeOH 95:5) to afford a fraction (1.4 g), which was re-subjected to repeated CC: *Fr. A* (120.5 mg), *B* (98.8 mg), *C* (50.5 mg), *D* (105.8 mg), and *E* (88.5 mg). Repeated CC of *Fr. B* (SiO_2; petroleum

ether/acetone 80:20) afforded compounds **1**, **2**, and **4** (25.2, 12.2, and 20 mg, resp.). *Fr. C* was re-subjected to CC (SiO_2; petroleum ether/acetone 90:10) to afford **5** and **6** (20.2 and 15.4 mg, resp.). *Fr. D* was also purified by CC (SiO_2; petroleum ether/acetone 84:16) to afford **3** and **7** (10.0 and 20.0 mg, resp.).

Leishmanicidal in vitro *Activity. L. major* (DESTO) promastigotes were grown at 22–25° in *RPMI-1640* (*Sigma*) medium containing 10% of heat-inactivated (56° for 30 min) fetal bovine serum. The promastigote culture in the logarithmic phase of growth was maintained, and the final concentration of parasites was adjusted to 2×10^6 parasites/ml. The test compound (1 mg) was dissolved in DMSO (50 µl), then the volume was made up to 1 ml with RPMI-1640. Test compound (20 µl) was added in the first well, which contained medium (180 µl), and was then serially diluted. Parasite suspension (100 µl) was added into each well of the 96-well plates, and incubated at 21–22° for 72 h, in the presence and absence of amphotericin B (pos. control). The experiments were carried out in duplicate, and the numbers of surviving parasites were counted in a *Neubauer* chamber. The 50%-inhibitory concentration (IC_{50}) was determined with EZ-Fit 5 software (*Perrella Scientific*).

16,24-Cyclo-13,14-secoergost-2-ene-18,26-dioic Acid 14:17,14:27-Diepoxy-5α,6β,11β,13,20,22-hexahydroxy-1,15-dioxo-γ-lactone δ-Lactone (=*(1*R,*2*S,*5*R,*7*S,*8*S,*9*R,*14*R,*15*R,*17*R,*18*S,*21*S,*24*R,*26*S,*27*R*)-5,7,14,15-Tetrahydroxy-2,9,26-trimethyl-3,19,23,28-tetraoxaoctacyclo[16.9.1.1^{18,27}.0^{1,5}.0^{2,24}.0^{8,17}.0^{9,14}.0^{21,26}]nonacos-11-ene-4,10,22,29-tetrone*; **1**). Yield: 25.2 mg (71 ppm). Colorless, amorphous powder. $[\alpha]_D^{25} = -211$ ($c = 3.1$, acetone). UV (MeOH): 226 (2.88). IR ($CHCl_3$): 3432 (OH), 1778 (lactone), 1670 (α,β-unsat. ketone). ^{1}H-NMR (400 MHz, CD_3OD): see *Table 1*. ^{13}C-NMR (100 MHz, CD_3OD): see *Table 2*. EI-MS: 560 (37.7, M^+), 558 (35.7), 544 (27.5), 526 (37.7), 498 (26.4), 482 (68), 171 (57.2), 147 (77.3), 133 (100), 109 (70), 91 (60.7), 55 (75.5). HR-EI-MS: 560.5457 (M^+, $C_{28}H_{32}O_{12}^+$; calc. 560.5465).

16,24-Cyclo-13,14-secoergosta-18,26-dioic Acid 5,6:14:17,14:27-Triepoxy-13,20,22-trihydroxy-3α-methoxy-1,15-dioxo-γ-lactone δ-Lactone (=*(1*R,*2*S,*5*R,*8*S,*9*R,*12*S,*14*R,*15*R,*17*R,*18*S,*21*S,*24*R,*26*S,*27*R*)-5,14-Dihydroxy-12-methoxy-2,9,15,26-tetramethyl-3,19,23,28-tetraoxaoctacyclo[16.9.1.1^{18,27}.0^{1,5}.0^{2,24}.0^{8,17}.0^{9,14}.0^{21,26}]nonacosane-4,10,22,29-tetrone*; **2**). Yield: 12.2 mg (34 ppm). Yellowish gum. $[\alpha]_D^{25} = -122$ ($c = 1.1$, CH_2Cl_2). UV (MeOH): 201 (2.68). IR ($CHCl_3$): 3348 (OH), 1776 (lactone). ^{1}H-NMR (400 MHz, $CDCl_3$): see *Table 1*. ^{13}C-NMR (100 MHz, $CDCl_3$): see *Table* 2. EI-MS: 558 (10.2, M^+), 540 (10.5), 508 (22.5), 452 (11.1), 403 (10.7), 375 (99.7), 171 (43.2), 157 (92.2), 147 (52.7) 133 (100), 105 (61.5), 91 (79.5), 67 (54.2), 55 (78.1). HR-EI-MS: 558.5740 (M^+, $C_{29}H_{34}O_{11}^+$; calc. 558.5736).

16,24-Cyclo-13,14-secoergost-2-ene-18,26-dioic Acid 14:17,14:27-Diepoxy-5α,13,20,22-tetrahydroxy-1,15-dioxo-γ-lactone δ-Lactone (=*(1*R,*2*S,*5*R,*8*S,*9*R,*14*R,*17*R,*18*S,*21*S,*24*R,*26*S,*27*R*)-5,14-Dihydroxy-2,9,26-trimethyl-3,19,23,28-tetraoxaoctacyclo[16.9.1.1^{18,27}.0^{1,5}.0^{2,24}.0^{8,17}.0^{9,14}.0^{21,26}]nonacos-11-ene-4,10,22,29-tetrone*; **3**). Yield: 10 mg (40 ppm). Colorless, amorphous powder. $[\alpha]_D^{25} = -20$ ($c = 1.8$, MeOH). UV (MeOH): 220 (2.56). IR ($CHCl_3$): 3434 (OH), 1779 (lactone), 1668 (α,β-unsat. ketone). ^{1}H-NMR (400 MHz, $CDCl_3$): see *Table 1*. ^{13}C-NMR (100 MHz, $CDCl_3$): see *Table 2*. EI-MS: 528 (17.4, M^+), 497 (37.5), 481 (24.9), 453 (16.0) 402 (63.2), 374 (81.9), 361 (23.6),321 (18.2), 220 (16.6), 188 (45.6), 171 (20.6), 156 (70.6), 132 (100), 121 (51.5), 104 (61.3), 91 (15.5), 79 (72.6), 67 (56), 55 (88.2). HR-EI-MS: 528.5465 (M^+, $C_{28}H_{32}O_{10}^+$, calc. 528.5477).

Physalin H (**4**). Yield: 20 mg (57 ppm). Colorless needles. M.p. 275–278°. $[\alpha]_D^{25} = -285$ ($c = 2.4$, acetone). UV (MeOH): 227 (2.58). IR ($CHCl_3$): 3434 (OH), 1779 (lactone), 1668 (α,β-unsat. ketone). ^{1}H-NMR (400 MHz, CD_3OD): 1.12 (*s*, Me(28)); 1.26 (*s*, Me(19)); 1.82 (*s*, Me(21)); 3.77 (*t*, *J*(27,27) = 14.5, H–C(27)); 3.81–3.83 (*m*, $W_{1/2} = 9.5$, H–C(6)); 4.33 (*dd*, *J*(27,27) = 14.9, *J*(27,25) = 4.4, H–C(27)); 4.35 (*t*, *J*(22,23) = 2.3, H–C(22)); 5.62 (*dd*, *J*(2,3) = 10.1, *J*(2,4β) = 2.6, H–C(2)); 6.52 (*ddd*, *J*(3,2) = 10.1, *J*(3,4α) = 4.9, *J*(3,4β) = 2.5, H–C(3)). ^{13}C-NMR (100 MHz, CD_3OD): 15.0 (C(19)); 22.0 (C(28)); 25.9 (C(12)); 25.9 (C(21)); 27.7 (C(11)); 31.9 (C(24)); 32.4 (C(9)); 33.2 (C(23)); 37.2 (C(7)); 38.3 (C(4)); 39.9 (C(8)); 51.4 (C(25)); 56.1 (C(10)); 56.4 (C(16)); 61.7 (C(27)); 74.4 (C(6)); 78.0 (C(13)); 78.7 (C(22)); 80.6 (C(17); 81.5 (C(5)); 82.1 (C(20)); 108.4 (C(14)); 128.6 (C(2)); 144.4 (C(3)); 170.0 (C(18)); 174.0 (C(26)); 204.1 (C(1)); 210.2 (C(15)). EI-MS: 562 (21.2, M^+) 544 (22.5), 526 (21.6), 508 (40.6), 454 (7.2), 171 (48.1), 147 (49.8) 133 (66.2), 109 (100), 91 (56.4), 55 (71.3). HR-EI-MS: 562.1920 ($C_{28}H_{31}ClO_{10}^+$, calc. 562.1925).

Isophysalin B (**5**). Yield: 20.2 mg (57 ppm). Colorless, amorphous powder. UV (MeOH): 227 (2.8). $[\alpha]_D^{25} = -85$ ($c = 3.5$, CH_2Cl_2). IR ($CHCl_3$): 3415 (OH), 1769 (lactone). ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.22 (*s*, Me(19)); 1.30 (*s*, Me(28)); 1.96 (*s*, Me(21)); 3.77 (*d*, *J*(27,27) = 14.5, H–C(27)); 4.50 (*t*, *J*(22,23) = 4.6, H–C(22)); 4.55 (*dd*, *J*(27,27) = 14.5, *J*(27,25) = 4.6, H–C(27)); 5.53–5.56 (*m*, H–C(3)); 5.66 (*dd*, *J*(6,7α) = 10.5, *J*(6,7β) = 2.6, H–C(6)); 6.01 (*d*, *J* = 3.4, H–C(4)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 19.2 (C(28)); 21.4 (C(19)); 24.6 (C(11)); 25.3 (C(12)); 25.9 (C(7)); 26.5 (C(21)); 31.2 (C(24)); 32.0 (C(9)); 33.0 (C(23)); 39.2 (C(8)); 39.5 (C(2)); 50.9 (C(25)); 55.1 (C(10)); 56.3 (C(16)); 60.7 (C(27)); 76.9 (C(22)); 79.9 (C(13)); 80.3 (C(20)); 81.0 (C(17)); 107.6 (C(14)); 121.2 (C(3)); 126.5 (C(4)); 128.8 (C(6)); 139.6 (C(5)); 166.5 (C(26)); 172.1 (C(18)); 208.2 (C(1)); 213.9

(C(15)). EI-MS: 510 (69.2, M^+), 492 (90.3), 464 (88.7), 171 (47.2), 131 (80.2) 105 (44.5), 131 (80.2), 105 (44.5), 91 (76.5), 67 (44.2). HR-EI-MS: 510.5319 (M^+, $C_{28}H_{30}O_9^+$; calc. 510.5324).

5β,6β-Epoxyphysalin B (**6**). Yield: 15.4 mg (43 ppm). Colorless solid. M.p. 263–265. UV (MeOH): 222 (2.4). $[\alpha]_D^{25} = -111$ ($c = 9.5$, CH_2Cl_2). IR ($CHCl_3$): 3415 (OH), 1779 (lactone), 1659 (α,β-unsat. ketone). ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.12 (*s*, Me(19)); 1.26 (*s*, Me(28)); 1.82 (*s*, Me(21)); 3.22 (*d*, $J(6\alpha,7\beta) = 4.6$, H–C(6)); 3.77 (*t*, $J(27,27) = 14.5$, H–C(27)); 4.33 (*dd*, $J(27,27) = 14.9$, $J(27,25) = 4.4$, H–C(27)); 4.35 (*t*, $J(22,23) = 2.3$, H–C(22)); 5.62 (*dd*, $J(2,3) = 10.1$, $J(2,4\beta) = 2.6$, H–C(2)); 6.52 (*ddd*, $J(3,2) = 10.1$, $J(3,4\alpha) = 4.9$, $J(3,4\beta) = 2.5$, H–C(3)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 19.5 (C(28)); 23.0 (C(19)); 23.5 (C(11)); 23.5 (C(21)); 24.8 (C(12)); 25.6 (C(7)); 31.0 (C(24)); 32.9 (C(23)); 34.2 (C(9)); 37.3 (C(8)); 38.4 (C(4)); 50.0 (C(10)); 50.8 (C(25)); 56.7 (C(16)); 59.7 (C(27)); 61.7 (C(5)); 64.8 (C(6)); 76.8 (C(22)); 79.8 (C(13)); 80.9 (C(20)); 81.0 (C(17)); 106.9 (C(14)); 127.7 (C(2)); 146.3 (C(3)); 172.1 (C(18)); 173.0 (C(26)); 205.8 (C(1)); 207.5 (C(15)). EI-MS: 526 (60.6, M^+), 497 (25.2), 427 (19.4), 402 (22.4), 390 (73.3), 171 (27.6), 159 (98.6), 135 (71.9) 109 (100), 91 (76.8), 67.1 (39.8), 55 (69.9). HR-EI-MS: 526.5310 (M^+, $C_{28}H_{30}O_{10}^+$; calc. 526.5318).

27-Hydroxy-1-oxowitha-5,14,24-trienolide (=*(6*R*)-3-(Hydroxymethyl)-4-methyl-6-[(1*S*)-1-[(6a*R*,7*E*,10-*S*,11*S*,13a*S*,13b*R*)-2,3,4,6,6a,9,10,11,12,13,13a,13b-dodecahydro-7,11,13b-trimethyl-1-oxo-1*H*-cyclonona[*a*]naphthalen-10-yl]ethyl]-5,6-dihydro-2*H*-pyran-2-one*; **7**). Yield: 20 mg (57 ppm). Yellowish gum. UV (MeOH): 201 (2.75). $[\alpha]_D^{25} = +113$ ($c = 0.8$, MeOH). IR ($CHCl_3$): 3411 (OH), 1715 (α,β-unsat. δ-lactone), 1657 (C=C). ^{1}H-NMR (400 MHz, $CDCl_3$): see *Table 1*. ^{13}C-NMR (100 MHz, $CDCl_3$): see *Table 2*. EI-MS: 438 (6.7, M^+), 410 (11.2), 194 (12.6), 168 (12.6), 155 (26.9), 135 (23.5), 124 (100), 109 (32.6), 95 (35.9), 81.0 (33.5), 67.0 (34.0), 55 (52.4). HR-EI-MS: 438.5978 (M^+, $C_{28}H_{38}O_7^+$, calc. 438.5989).

REFERENCES

[1] B. Makino, M. Kawai, K. Kito, H. Yamamura, Y. Butsugan, *Tetrahedron* **1995**, *51*, 12529.
[2] I. Kirson, E. Glotter, D. Lavie, *J. Chem. Soc. C* **1971**, *11*, 2032.
[3] E. Glotter, I. Kirson, A. Abraham, P. D. Sethi, S. S. Subramanian, *J. Chem. Soc., Perkin Trans. 1* **1974**, *4*, 1370.
[4] E. M. Choi, J. K. Hwang, *J. Ethanopharmacol*. **2003**, *89*, 171.
[5] J. Q. Gu, W. Li, Y. H. Kang, B. N. Su, H. H. Fong, R. B Van Breemen, J. M. Pezzuto, A. D. Kinghorn, *Chem. Pharm. Bull.* **2003**, *51*, 530.
[6] Y. S. Lin, H. C. Chiang, W. S. Kan, E. Hone, S. J. Shih, M. H. Won, *Am. J. Chin. Med.* **1992**, *20*, 233.
[7] M. B. Soares, M. C. Bellintani, I. M. Ribeiro, T. C. Tomassini, D. S. R. Ribeiro, *Phytother. Res.* **2002**, *16*, 445.
[8] R. C. Pietro, S. Kashima, D. N. Sato, A. H. Januario, S. C. Franca, *Phytomedicines* **2000**, *7*, 335.
[9] A. H. Januario, E. R. Filho, R. C. Pietro, S. Kashima, D. N. Sato, S. C. Franca, *Phytother. Res.* **2002**, *16*, 445.
[10] R. R. Roman, F. Alarcon-Aguilar, A. Lara-Lemus, J. L. Flores-Saenz, *Arch. Med. Res.* **1992**, *23*, 59.
[11] A. Caceres, H. Menendez, E. Mendez, E. Cohobon, B. E. Samayoa, E. Jauregui, E. Peralta, G. Carrillo, *J. Ethanopharmacol.* **1995**, *48*, 85.
[12] R. R. Alluri, R. J. Miller, W. H. Shelver, S. K. W. Khalil, *Lloydia* **1976**, *39*, 405.
[13] M. J. Chan-Bacab, L. M. Pefia-Rodriguez, *Nat. Prod. Rep*. **2001**, *18*, 674.
[14] B. Hazra, A. K. Saha, R. Ray, D. K. Roy, P. Sur, A. Banerjee, *Trans. R. Soc. Trop. Med. Hyg*. **1987**, *81*, 738.
[15] A. T. Evans, S. L. Croft, *Phytother. Res.* **1987**, *1*, 25.
[16] C. W. Wright, J. D. Phillipson, *Phytother. Res.* **1990**, *4*, 127.
[17] R. Sunayama, M. Kuroyanagi, K. Umehara, A. Ueno, *Phytochemistry* **1993**, *34,* 529.
[18] A. I. Scott, 'Interpretation of UV Spectra of Natural Products', Pergamon Press, Oxford, 1964, p. 30.
[19] B. Makino, M. Kawai, Y. Iwata, H. Yamamura, Y. Butsugan, K. Ogawa, M. Hayashi, *Bull. Chem. Soc. Jpn*. **1995**, *68*, 219.
[20] M. I. Choudhary, S. A. A. Shah, S. G. Musharraf, F. Shaheen, Atta-ur-Rahman, *Nat. Prod. Res.* **2003**, *17*, 215.
[21] H. E. Gottlieb, M. Cojocaru, S. C. Sinha, M. Saha, A. Bagchi, A. ALi, A. B. Ray, *Phytochemistry* **1987**, *26*, 1801.
[22] T. Matsuura, M. Kawai, R. Nakashima, Y. Butsugan, *Tetrahedron Lett*. **1969**, *14*, 1083.
[23] M. Kawai, T. Matsuura, T. Taga, K. Osaki, *J. Chem. Soc. B* **1970**, 812.
[24] P. Lee, Y. Kitamura, K. Kaneko, M. Shiro, G-J Xu, Y-P Chen, H-Y Hsu, *Chem. Pharm. Bull*. **1988**, *36*, 4316.
[25] Atta-ur-Rahman, M. Shabbir, M. Yousuf, S. Qureshi, Dur-e-Shahwar, A. Naz, M. I. Choudhary, *Phytochemistry* **1999**, *52*, 1361.

[26] M. I. Choudhary, S. Yousuf, S. A. Nawaz, S. Ahmed, Atta-ur-Rahman, *Chem. Pharm. Bull.* **2004**, *52*, 1358.
[27] S. Nagafuji, H. Okabe, H. Akahane, F. Abe, *Biol. Pharm. Bull.* **2004**, *27*, 193.
[28] B. Makino, M. Kawai, T. Ogura, M. Nakanishi, H. Yamamura, Y. Butsugan, *J. Nat. Prod*. **1995**, *58*, 1668.
[29] L. R. Row, N. S. Sarma, K. S. Reddy, T. Matsuura, R. Nakashima, *Phytochemistry* **1978**, *17*, 1647.

Received March 1, 2005