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Dihydrophenanthrenes and other antifungal stilbenoids from *Stemona* cf. *pierrei*

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Abstract

Three new dihydrophenanthrenes, stemanthrenes A–C, along with the new dihydrostilbene stilbostemin G were isolated and identified from the underground parts of *Stemona* cf. *pierrei* together with the known pinosylvin, 4′-methylpinosylvin, dihydropinosylvin, stilbostemins B, D, and E as well as the pyrrolo[1,2-a]azepine alkaloids protostemonine and stemonine. The structures of all new stilbenoids, elucidated by NMR analyses, showed a common substitution pattern for aromatic ring A and characteristic C-methylations for ring B. The trivial name racemosol, previously reported for *S. collinsae*, was renamed to stemanthrene D due to its priority for another compound. Bioautographic tests on TLC plates with *Cladosporium herbarum* displayed high antifungal activity for compounds with an unsubstituted aromatic ring A, e.g. pinosylvin, but only weak effects for the higher substituted stilbostemin G and stemanthrenes A–C. Similar results were obtained by germ tube inhibition of five microfungi using 2-fold serial broth dilutions determined by a microplate reader. Because of weak inhibition and chemical instability of stemanthrenes, no EC_{50} and EC_{90} values could be calculated.

Keywords: *Stemona* cf. *pierrei*; Stemonaceae; Stilbenoids; Dihydrophenanthrenes; Stemanthrenes A–C; Dihydrostilbenes; Stilbostemin G; Antifungal; Bioautography; Microdilution; Protostemonine; Stemonine; Pyrrolo[1,2-a]azepines; Stemona alkaloids

1. Introduction

In connection with our current screening for biologically active compounds from Stemonaceae (Pacher et al., 2002; Brem et al., 2002; Kaltenegger et al., 2003) *Stemona* cf. *pierrei* Gagnep. was collected in East Thailand and tested for antifungal activity. It is a small climber with tuberous roots growing in open scrub vegetation characterized by angular stems and small dark purple flowers with conspicuous yellow appendices of the thecae (Fig. 1). Since the latter, rather typical morphological character was not mentioned in the original description by Gagnepain (1934), "cf." was inserted before the species name indicating its uncertain taxonomic position. Corresponding to our previous findings of *S. collinsae* Craib (Pacher et al., 2002), various stilbenoids were shown to form clear inhibition spots on bioautographic tests on TLC plates against the fungus *Cladosporium herbarum* (Pers.: Fr.) Link. However, in contrast to *S. collinsae* and many other *Stemona* species already analysed in our laboratory (Hartl, 2003; Kaltenegger, 2003), the HPLC profile from the methanolic crude extract of the underground parts deviated by a preponderance of various dihydrophenanthrenes (**1**–**3**). This was remarkable since only one derivative, racemosol (Sekine et al., 1997), was known so far from the genus, meanwhile found in several species (Pacher et al., 2002; Hartl, 2003). Altogether ten stilbenoids were detected in the methanolic root extract of *S.* cf. *pierrei* from which four proved to be hitherto undescribed compounds: the dihydrophenanthrenes stemanthrenes A-C (**1**–**3**), and the dihydrostilbene stilbostemin G (**4**). In addition, the already known dihydrostilbenes stilbostemins B (**5**) and D (**6**) (Zhao et al., 1995; Pacher et

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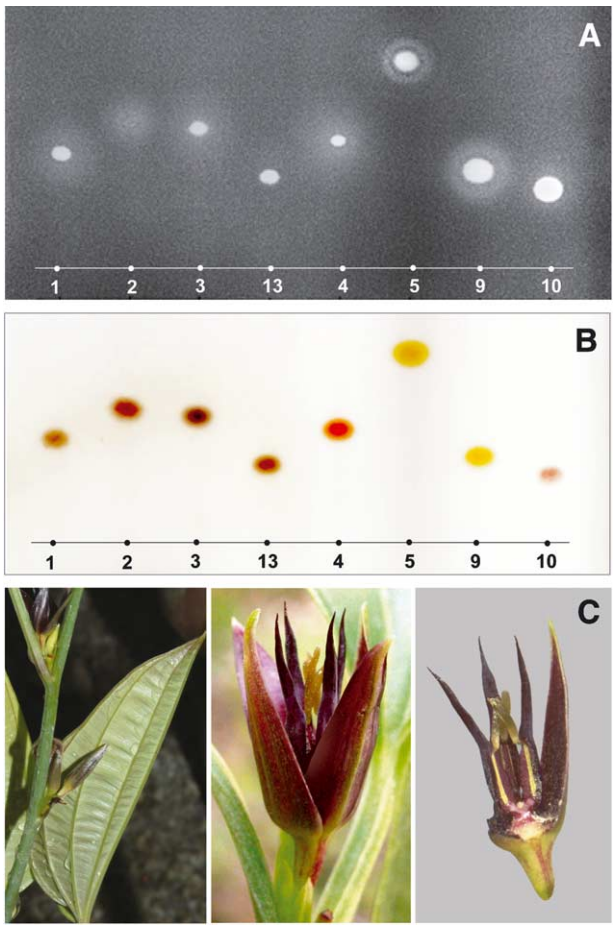


Fig. 1. A: Bioautographic comparison of different antifungal activities of dihydrophenanthrenes (**1**–**3**, **13**), dihydrostilbenes (**4**, **5**, **9**), and stilbene (**10**) by direct spraying of TLC plates with a conidial suspension of *Cladosporium herbarum*. (see Exp.section). Solvent = hexane–Et_2O (6:4). B: Colour properties of the same compounds sprayed with MeOH–HOAc–H_2SO_4–anisaldehyde-reagent (85:10:8:0.5). C: Morphological features of *S.* cf. *pierrei*. Yellow appendices on the top of the thecae of the dark purple stamens represent typical floral characters.

al., 2002) could also be isolated for structure elucidation, whereas stilbostemin E (**7**), dihydropinosylvin (**8**), pinosylvin (**9**), and 4′-methylpinosylvin (**10**) were identified as minor compounds by HPLC-UV-comparison with authentic samples.

As expected from previous chemical reports on *Stemona* species (Pilli and Ferreira de Oliveira, 2000; Xu, 2000; Brem et al., 2002; Kaltenegger et al., 2003), characteristic pyrrolo[1,2-a]azepine alkaloids could also be detected in *S.* cf. *pierrei* from which the known protostemonine (**11**) (Irie et al., 1970; Ye et al., 1994) and stemonine (**12**) (Koyama and Oda, 1970; Sakata et al., 1978) were isolated and identified by NMR analyses as main components. In the present paper we report the isolation and structure elucidation of the new stilbenoids (**1**–**4**) and discuss their antifungal activities observed by bioautography on TLC plates and germ tube inhibition tests in microwells with 2-fold serial broth dilutions.

2. Results and discussion

Methanolic extracts of air-dried underground parts, including roots and rhizomes, of four different individuals of *S.* cf. *pierrei* were concentrated and partitioned between water and chloroform. Subsequent HPLC-UV analyses of the lipophilic fractions showed largely uniform chemical profiles with only minor quantitative variations. Thus the fractions were combined and used for preparative MPLC and TLC. As already indicated in our foregoing communication (Pacher et al., 2002), chromatographic separations of some stilbenoids were difficult and pure compounds could only be obtained by crystallization. Compared with the already known stilbenoids (Pacher et al., 2002), *S.* cf. *pierrei* deviated by a series of derivatives with characteristic maxima at 282 and 216 nm (MeOH) in the UV spectra. Close structural affinity between the three main components (**1**–**3**) was suggested by IR spectra with prominent signals at 1614–1576 cm^{-1} and 1490–1424 cm^{-1} as well as typical double bands for two phenolic OH groups at 3604–3534 cm^{-1} ($CHCl_3$). A similar IR spectrum was also observed for compound **4** which, however, clearly deviated by less intense absorption bands in the UV spectrum typical for dihydrostilbenes (see Pacher et al., 2002).

Some ^1H and ^{13}C NMR resonances of compounds **1**–**3** were almost identical indicating the same subunits within the series. This was especially clear for the ^1H and ^{13}C data of atoms no. 1–6, 1″-2″, 2-OMe, and 3-OH (Table 1). Supported by NOESY and HMBC experiments the structures **1**–**3** were identified as dihydrophenanthrenes with identical A-ring units with OCH_3 in position 2 and OH in position 3. The protons at positions 4 and 5 appeared as characteristic aromatic dublets with typical *ortho* coupling constants (Table 1). In the case of the A-ring strong NOE contacts 2-OMe to 1″-H_2 and 3-OH, and a weak contact 3-OH to 4-H were characteristic for 2-methoxy-3-hydroxy substitution of compounds **1**–**3**. The positions of the remaining substituents on the B-rings of **1**–**3** were also determined by NOESY and HMBC experiments. In the case of compound **1** the NOE contact chain 2″-H_2↔2′-H, 2′H↔3′-OH, 3′-OH↔4′-CH_3, 4′-CH_3↔5′-OCH_3, and 5′-OCH_3↔5-H allowed the unambigous identification of a 3′-hydroxy-4′-methyl-5′-methoxy substituted B-ring. The B-ring of compound **2** was also characterized by a hydroxy, a methoxy and a methyl group and was therefore an isomer of **1**. NOESY crosspeaks between 2′-H and the methoxy protons, and between 5-H and the hydroxy group of the B-ring indicated 3′-methoxy-4′-methyl-5′-hydroxy substitution. C/H long range correlation (HMBC) crosspeaks from 5′-OH to C-4′, C-5′, and C-6′ and from 4′-CH_3 to C-3′, C4′, and C-5′ confirmed this substitution pattern and allowed also the assignment of the quaternary ^{13}C resonances. Compound **3** showed an additional methyl-group at the

Table 1
^{1}H and ^{13}C NMR data of stemanthrenes A-C (**1–3**) and stilbostemin G (**4**) in $CDCl_3$

	^{1}H NMR				^{13}C NMR			
No.	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
1					130.8 *s*	131.5 *s*	130.5 *s*	134.8 *s*
2					143.5 *s*	144.4 *s*	143.2 *s*	145.3 *s*
3					147.0 *s*	147.0 *s*	146.9 *s*	149.0 *s*
4	6.87 *d* (8.7)	6.90 *d* (8.5)	6.85 *d* (8.8)	6.83 *dd* (7.8, 1.5)	113.1 *d*	113.0 *d*	113.0 *d*	113.6 *d*
5	8.05 *d* (8.7)	7.72 *d* (8.5)	8.02 *d* (8.8)	6.96 *dd* (7.8, 7.8)	124.0 *d*	122.3 *d*	124.3 *d*	124.8 *d*
6				6.76 *dd* (7.8, 1.5)	126.2 *s*	126.3 *s*	126.6 *s*	121.4 *d*
1′					137.5 *s*	136.9 *s*	136.1 *s*	140.6 *s*
2′	6.51 *s*	6.43 *s*		6.35 *d* (1.3)	110.5 *d*	102.8 *d*	116.2 *s*	108.0 *d*
3′					152.9 *s*	156.6 *s*	151.3 *s*	154.3 *s*
4′					116.1 *s*	110.6 *s*	114.9 *s*	109.5 *s*
5′					156.9 *s*	151.3 *s*	154.5 *s*	158.5 *s*
6′				6.31 *d* (1.3)	120.1 *s*	114.7 *s*	120.1 *s*	103.5 *d*
1″	2.79 *m*	2.79 *m*	2.77 *m*	2.91 *m*	29.6 *t*	30.0 *t*	25.9 *t*	31.5 *t*
2″	2.67 *m*	2.70 *m*	2.69 *m*	2.84 *m*	22.4 *t*	22.7 *t*	21.9 *t*	36.7 *t*
2-OMe	3.81 *s*	3.80 *s*	3.82 *s*	3.78 *s*	61.4 *q*	61.4 *q*	61.4 *q*	61.3 *q*
3-OH	5.61 *s*	5.66 *s*	5.61 *s*	5.57 *v.br.s*				
2′-Me			2.21 *s*				11.8 *q*	
3′-OH/OMe	4.70 *br.s* (OH)	3.85 *s* (OMe)	4.71 *s* (OH)	4.67 *v.br.s* (OH)		55.7 *q*		
4′-Me	2.22 *s*	2.16 *s*	2.24 *s*	2.08 *s*	8.7 *q*	8.1 *q*	8.9 *q*	7.8 *q*
5′-OH/OMe	3.53 *s* (OMe)	5.36 *s* (OH)	3.50 *s* (OMe)	3.79 *s* (OMe)	59.6 *q*		59.9 *q*	55.7 *q*

B-ring, however, all other ^{1}H and ^{13}C chemical shifts correlated very well with the ones of compound **1**. The NOESY contact chain 2″-H_2↔2′-CH_3↔3′-OH↔4′-CH_3↔5′-OCH_3↔5-H was conclusive for a 3′-hydroxy-2′,4′-dimethyl-5′-methoxy substituted B-ring of compound **3**. Compound **3** is therefore comparable to compound **1** with an additional methyl group at position 2′. A combination of C/H correlation (HMQC), C/H long range correlation (HMBC) and NOESY data allowed the unambiguous assignment of all ^{13}C resonances of compounds **1–3** (Table 1). Complete lists of all NOESY and HMBC cross peaks are given in the Experimental section. It should be mentioned that the phenolic OH resonances of all stemanthrenes showed only very moderate line broadening and could be fully used in the NOESY and HMBC correlations. The high resolution EI-MS results for compounds **1–3** were also fully compatible with the proposed structures (see Experimental section).

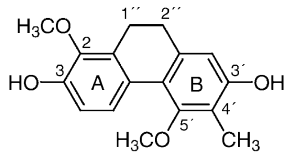
Compound **4** was a dihydrostilbene of the type we have reported recently for *S. collinsae* (Pacher et al., 2002) with OCH_3 and OH at one ring and OCH_3, OH, and CH_3 at the other. NMR data and high resolution EI-MS were compatible with this general structure. The ^{1}H and ^{13}C NMR resonances of one aromatic ring of **4** showed almost identical features with the A-ring of stilbostemin E (**7**) of the paper cited above. This was confirmed by the strong NOESY cross peak between 2-OCH_3 and 1″-H_2, leaving only position 3 for the OH group and positions 4, 5, and 6 for the aromatic ABC spin system with a characteristic combination of *ortho* and *meta* coupling constants (Table 1). The 2-methoxy-3-hydroxy substitution of the A-ring of dihydrostilbene 4 was therefore the same as in stemanthrenes **1–3**. The substitution pattern of the B-ring was derived from NOESY and HMBC data. Since the OH resonances were very broad, they could not be used for the 2D techniques. However, NOESY data allowed the identification of the 5′-OCH_3 group due to the cross peaks between 5′-OCH_3 and 6′-H and between 6′-H and the methylene bridge protons 1″-H_2/2″-H_2. The second proton at ring B was allocated to position 2′. This could be proved by NOESY contacts between 2′-H and the bridge protons 1″-H_2/2″-H_2, and was further supported by a small *meta* coupling of $J = 1.3$ Hz for the protons 2′-H and 6′-H. The identification of position 3′ for OH and position 4′ for CH_3 followed from analysis of the HMBC cross peaks which proved that the methyl group was placed between two oxygen substituted carbon atoms: 4′-CH_3→C-3′ ($\delta = 154.3$ ppm), C-4′, and C-5′ ($\delta = 158.5$ ppm) (a complete set of HMBC data is listed in the Experimental part). The 3′-hydroxy-4′-methyl-5′-methoxy substitution of compound **4**, named stilbostemin G, is therefore identical with the substitution pattern of compound **1**.

The NMR spectra of **5** and **6** were identical with those of the two known dihydrostilbenes stilbostemin B (**5**) and D (**6**) which both appear to be ubiquitous in the genus *Stemona* and were already described for *S. tuberosa* Lour. (Zhao et al., 1995) and *S. collinsae* (Pacher et al., 2002). Meanwhile they were also detected in several other species together with **7** and **9** as well as the corresponding stilbenes 4′-methylpinosylvin **8** and pinosylvin **10** (Hartl, 2003; Kaltenegger, 2003). The four latter

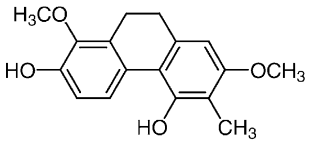
compounds were also detected in *S.* cf. *pierrei* by HPLC-UV-comparison with authentic samples. Aromatic C-methylation of stilbenoids was shown to be a typical chemical feature of *Stemona* (Pacher et al., 2002) and in fact, all derivatives of the present investigation, except the three minor compounds **7**, **9**, and **10**, were characterized by C-methylation of ring B. However, *S.* cf. *pierrei* can be distinguished from the other species by two additional chemical trends: (a) the predominant formation of dihydrophenanthrenes and (b) a preferred substitution pattern of ring A consisting of a methoxy and hydroxy group in *ortho* position (**1**–**4**, **7**).

The conversion from dihydrostilbenes to dihydrophenanthrenes was already suggested for stilbostemin F to racemosol (**13**) in *S. collinsae* (Pacher et al., 2002), and is known from Dioscoreaceae (Fritzemeier et al., 1984) and Orchidaceae (Fritzemeier and Kindl, 1983; Gehlert and Kindl, 1991). Therefore, the dihydrophenanthrene stemanthrene A (**1**) from *S.* cf. *pierrei* most probably can directly be derived from the new

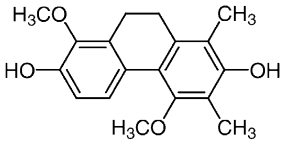
Stilbenoids



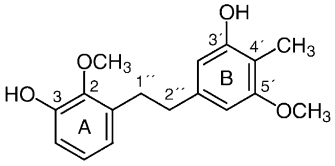
1 stemanthrene A



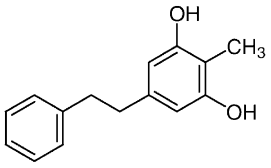
2 stemanthrene B



3 stemanthrene C



4 stilbostemin G



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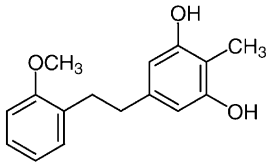
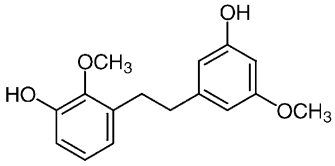
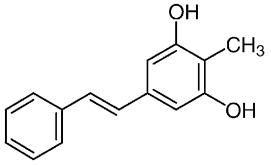


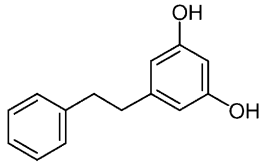
6 stilbostemin D



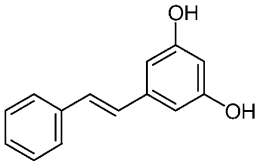
7 stilbostemin E



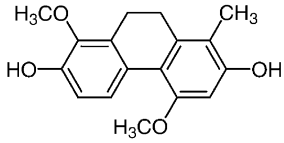
8 4′-methylpinosylvin



9 dihydropinosylvin

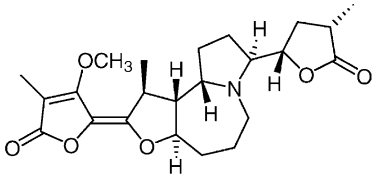


10 pinosylvin

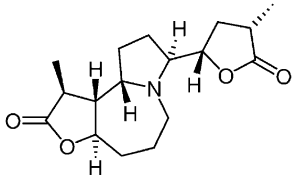


13 stemanthrene D (=racemosol)

Alkaloids



11 protostemonine



12 stemonine

dihydrostilbene stilbostemin G (**4**) with the same substitution patterns in the aromatic rings A and B. However, it was of special chemosystematic interest that the corresponding isomeric pair stilbostemin F and racemosol (**13**) known from *S. collinsae* and several other species (Hartl, 2003), was not detected in *S.* cf. *pierrei*. Thus, the insertion of a methyl group either at C-4′ or C-2′ position of ring B appears to be controlled by specific enzymatic activity.

Based on more detailed literature search it turned out that the trivial name racemosol for the dihydrophenanthrene **13** (Sekine et al., 1997) cannot longer be justified since it was already used for a prenylated stilbenoid isolated from *Bauhinia racemosa* Lamk. 11 years ago (Anjaneyulu et al., 1986). Moreover, the designation of **13** as racemosol was attributed to its original isolation from roots of *Asparagus racemosus* Willd. (Asparagaceae) (Sekine et al., 1997), which, however, most likely has been confused with those of a *Stemona* species (see also Pacher et al., 2002; Brem et al., 2002; Kaltenegger et al., 2003). A second "irregular" racemosol was also published for a 4-phenylcoumarin isolated from *Mesua racemosa* (Planch. ex Triana et Planch.) Kosterm. (Clusiaceae) (Morel et al., 1999). Consequently racemosol from *Stemona* species has to be renamed. With respect to its closely related isomer stemanthrene A (**1**) we therefore suggest to name it stemanthrene D (**13**).

Based on TLC *Cladosporium* bioassay (bioautography) antifungal properties could only be observed for stilbenoids. The alkaloids protostemonin (**11**) and stemonin (**12**), however, were already known for their insect toxicity (Sakata et al., 1978; Kaltenegger et al., 2003). Bioautography with seven stilbenoids from *S.* cf. *pierrei* (**1**–**5**, **9**, **10**) permitted a preliminary overview of their antifungal activities in comparison to those isolated from *S. collinsae* (Pacher et al., 2002). According to that, the antifungal capacities of the dihydrophenanthrenes **1**–**3**, **13** and stilbostemin G (**4**) appeared generally weaker than those of the more widespread dihydrostilbenes **5**, **9**, and stilbene **10** (Fig. 1; see also Pacher et al., 2002). Of special interest, however, was the difference between the two isomers stemanthrene A (**1**) and B (**2**). In this case **1** showed a small but clearly visible inhibition zone, similar to dihydrophenanthrenes **3** and **13**, whereas the corresponding zone of **2** was completely overgrown with the fungus suggesting only very weak or even no activity. To compare the activities with previous results, germ tube inhibition tests in microwells were carried out against five microfungi, already used in our foregoing investigation (Pacher et al., 2002). According to that, the present data confirmed the higher antifungal capacity of some dihydrostilbenes and stilbenes, but showed only weak activity for dihydrophenanthrenes (Table 2). This was in contrast to Orchidaceae, where dihydrostilbenes were shown to be only precursors for the active dihydrophenanthrenes, accumulated as ecologically important phytoalexins (Fritzemeier and Kindl, 1983). As shown in Table 2, particularly high activity was observed for the stilbene pinosylvin (**10**), already well-known for its antifungal properties and role as phytoalexin (Gehlert et al., 1990). The corresponding dihydropinosylvin (**9**) and especially the higher substituted stilbostemin G (**4**) were clearly less active. Because of the weak germ tube inhibition of the stemanthrenes A-C (**1**–**3**), which was only around 10% along a concentration range of 0.8 to 200 μg/ml, a comparative probit log analysis was not possible (Table 2). Moreover, a change of colour to reddish brown was observed for the colourless solution of stemanthrene A (**1**) in contact with the spore suspension of *Pyricularia grisea* (Cooke) Sacc. indicating chemical instability. In fact, a renewed NMR analysis of originally pure **1** showed a high degree of decomposition. However, due to shortage of material a repetition of bioassays with freshly isolated stemanthrenes was not possible. It remains also open at present whether the four pinosylvin derivatives (**5**, **8**, **9**, **10**) may act as phytoalexins after fungal infection at least in young plant material as reported for pinosylvin (**10**) in *Pinus sylvestris* L. (Gehlert et al., 1990).

3. Experimental

3.1. General

NMR: Bruker DRX400 WB ($CDCl_3$ ^1H δ 7.26; ^{13}C δ 77.0). MS: Finnigan MAT 900 S. IR: Perkin-Elmer 16PC FT-IR. UV: Hewlett Packard, 8452A Diode Array Spectrophotometer. HPLC: Hewlett Packard 1090 Series II, UV diode array detection at 230 nm, column 150 (4.6 mm, Phenomenex Synergi Polar-RP80A, 4 μm, mobile phase acetonitrile (gradient 20–60%) in aqueous buffer (0.015 M *o*-phosphoric acid, 0.0015 M tetrabutylammonium hydroxide, pH 3), flow rate 1 ml/min.

3.2. Plant material

Underground parts (roots and rhizomes) of *S.* cf. *pierrei* were collected in East Thailand, Sri Sa Ket province, between Sri Sa Ket and Surin in open scrub (dipterocarp) vegetation. Voucher specimens (HG 910) are deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

3.3. Extraction and isolation

Underground parts of four individuals of *S.* cf. *pierrei* were air-dried for 3–4 weeks, ground separately and extracted twice with MeOH at room temp for 5 days, filtered and conc. The aqueous residues were extracted

Table 2
Germ tube inhibtion effect of stilbenoids from *Stemona* cf. *pierrei* against five microfungi determined with a microplate reader[a]

	Pyricularia grisea				*Cladosporium herbarum*				*Fusarium avenaceum*			
µg/ml	EC_{50}	(95% FL)	EC_{90}	(95% FL)	EC_{50}	(95% FL)	EC_{90}	(95% FL)	EC_{50}	(95% FL)	EC_{90}	(95% FL)
Pinosylvin (**10**)	7	(3–9)	16	(13–20)	10	(6–16)	58	(31–177)	36	(34–39)	50	(47–56)
Dihydropinosylvin (**9**)	44	(37–46)	55	(52–63)	32	(18–67)	209	(92- > 200)	56	(42–74)	131	(94–244)
Stilbostemine G (**4**)	> 200[b]		> 200[b]		71[b]		> 200[b]		48	(29–96)	> 200	(> 200)
Stemanthrenes A–C (**1**–**3**)	n.d.[c]		n.d.[c]		n.d.[c]		n.d.[c]		n.d.[c]		n.d.[c]	

	Alternaria citri				*Botrytis cinerea*			
µg/ml	EC_{50} (95% FL)		EC_{90} (95% FL)		EC_{50} (95% FL)		EC_{90} (95% FL)	
Pinosylvin (**10**)	35	(24–55)	46	(36–147)	11	(8–14)	39	(26–70)
Dihydropinosylvin (**9**)	89[b]	> 200[b]			69[b]		77[b]	
Stilbostemine G (**4**)	144[b]	> 200[b]			> 200[b]		> 200[b]	
Stemanthrenes A–C (**1**–**3**)	n.d.[c]	n.d.[c]			n.d.[c]		n.d.[c]	

[a] 100 µl of stock solution (2 mg of test compound/250 µl of acetone/4.75 ml of 4% malt extract w/v, emulgated with 0.2% tween 80) were 2-fold microdiluted in 4% malt extract broth (w/v); negative control, stock solution without test compound. 50 µl of spore suspension (10^4 CFU/ml, 4% malt extract broth w/v) were added per well. An absorbance-blank at 620 nm was measured after 24 h incubation in darkness at room temperature. After 48 h fungal growth was determined by absorbance readings (620 nm) in the microwells. The percentage of inhibition compared to the negative control was determined from the calculation: (stock solution−blank of stock solution)/(negative control−blank of negative control)×100. Probit-log estimates were calculated from a concentration range of 200–0.1 µg/ml. FL, fiducial limits.

[b] No fiducial limits could be obtained because of weak germ tube inhibition.

[c] n.d., not determined; because of very weak germ tube inhibition of 10% along a concentration range of 0.8 to 200 µg/ml, a probit log analysis was not possible.

with $CHCl_3$ and the conc. $CHCl_3$ fractions were used for comparative HPLC and TLC, sprayed with MeOH–HOAc–H_2SO_4–anisaldehyde reagent (85:10:8:0.5) as well as bioautography on TLC plates. For preparative isolation the extracts from four individuals (68 g) were pooled together to give 800 mg crude extract which was roughly separated by column chromatography (Merck Si gel 60, 35–70 mesh) with solvent mixtures of hexane, EtOAc and MeOH. The stilbenoid containing fractions eluted with 30–70% EtOAc in hexane were further separated by preparative MPLC (400×40 mm column, Merck LiChroprep silica 60, 25–40 µm, UV detection, 254 nm) using mixtures of 10% and 15% EtOAc in hexane. Fractions eluted with 10% EtOAc in hexane afforded 8 mg of compound **5**, 8 mg of impure **8**, and 33 mg **6** as well as 80 mg of a mixture leading to 10 mg **2** and 14 mg **3** by a second MPLC separation and subsequent crystallization. 15% EtOAc in hexane was used to elute 8 mg impure **9**, 15 mg of **7**/**10**, and 22 mg of a mixture, which was purified by prep. TLC ($CHCl_3$-MeOH = 97:3) to give 9 mg **4**. Further elution with 15% EtOAc in hexane afforded 9 mg pure **1** by crystallization and again 3 mg of impure **4**. The more polar, alkaloid containing column fractions eluted with 10% MeOH in EtOAc were combined and separated by prep. MPLC using 100% EtOAc. The fractions afforded 4 mg **12** and 5 mg **11** purified by prep. TLC (15% MeOH in EtOAc).

3.4. Stemanthrene A. 2,7-Dihydroxy-1,5-dimethoxy-6-methyl-9,10-dihydrophenanthrene (**1**)

Colourless crystals, mp 130–132 °C. UV λ^{MeOH} 297sh, 282, 271sh, 216 nm. IR ν^{CHCl_3} cm^{-1} 3686 w, 3598 m, 3534 m, 3332 br, 2938 m, 2836 w, 1608 m, 1488 s, 1460 s, 1434 m, 1400 m, 1378 w, 1348 w, 1314 m, 1284 s, 1174 w, 1162 m, 1132 w, 1092 s, 1062 w, 1032 w, 1066 w, 972 w, 938 w, 882 w. ^1H and ^{13}C NMR see Table 1. NOESY: 2-OCH_3↔3-OH; 3-OH↔4-H (w); 4-H↔5-H; 5-H↔5′-OCH_3; 5′-OCH_3↔4′-CH_3; 4′-CH_3↔3′-OH; 3′-OH↔2′-H; 2′-H↔2″-H_2; 2″-H_2↔1″-H_2; 1″-H_2↔2-OCH_3. HMBC (H→C) 2-OCH_3→2; 3-OH→2, 3, 4; 4-H→2, 3, 6; 5-H→1, 3, 6′; 2′-H→3′, 4′, 6′; 3′-OH→2′, 3′, 4′; 4′-CH_3→3′, 4′, 5′; 5′-OCH_3→5′; 1″-H_2→1, 2, 6, 1′, 2″; 2″-H_2→1, 1′, 2′, 6′, 1″. EIMS (70 eV) *m*/*z* (%) = 286 (100, M^+), 271 (7), 239 (61), 143 (7), 115 (6). HREIMS *m*/*z* 286.1201 (calcd for $C_{17}H_{18}O_4$, 286.1205).

3.5. Stemanthrene B. 2,5-Dihydroxy-1,7-dimethoxy-6-methyl-9,10-dihydrophenanthrene (**2**)

Colourless crystals, mp 198–200 °C. UV λ^{MeOH} 309sh, 282, 273sh, 218 nm. IR ν^{CHCl_3} cm^{-1} 3694 w, 3598 w, 3538 m, 3304 br, 2958 m, 2840 w, 1614 m, 1576 w, 1486 s, 1466 s, 1432 m, 1406 w, 1380 w, 1354 w, 1316 m, 1284 m, 1178 w, 1162 w, 1138 s, 1112 m, 1056 w, 1026 w, 1010 w, 994 w, 964 w, 942 w, 884 w, 838 w. ^1H and ^{13}C

NMR see Table 1. NOESY: 2-OCH_3↔3-OH; 3-OH↔4-H (w); 4-H↔5-H; 5-H↔5′-OH; 5′-OH↔4′-CH_3; 4′-CH_3↔3′-OCH_3; 3′-OCH_3↔2′-H; 2′-H↔2″-H_2; 2″-H_2↔1″-H_2; 1″-H_2↔2-OCH_3. HMBC (H→C) 2-OCH_3→2; 3-OH→2, 3, 4; 4-H→2, 3, 6; 5-H→1, 3, 6′; 2′-H→3′, 4′, 6′; 3′-OCH_3→3′; 4′-CH_3→3′, 4′, 5′; 5′-OH→4′, 5′, 6′; 1″-H_2→1, 2, 6, 1′, 2″; 2″-H_2→1, 1′, 2′, 6′, 1″. EIMS (70 eV): *m*/*z* (%) = 286 (100, M^+), 271 (23), 253 (8), 143 (8), 115 (7). HREIMS *m*/*z* 286.1202 (calcd for $C_{17}H_{18}O_4$, 286.1205).

3.6. Stemanthrene C. 2,7-Dihydroxy-4,8-dimethoxy-1,3-dimethyl-9,10-dihydrophenanthrene (*3*)

Colourless crystals, mp 169–171 °C. UV λ^{MeOH} 298sh, 282, 270sh, 216 nm. IR ν^{CHCl_3} cm^{-1} 3688 w, 3604 m, 3534 m, 3380 br, 2938 w, 2834 w, 1604 m, 1580 w, 1490 s, 1476 m, 1448 w, 1424 m, 1406 w, 1350 w, 1314 w, 1284 s, 1168 w, 1150 w, 1106 s, 1082 w, 1020 w, 1002 w, 990 w, 972 w, 944 w, 930 w, 858 w. ^1H and ^{13}C NMR see Table 1. NOESY: 2-OCH_3↔3-OH; 3-OH↔4-H (w); 4-H↔5-H; 5-H↔5′-OCH_3; 5′-OCH_3↔4′-CH_3; 4′-CH_3↔3′-OH; 3′-OH↔2′-CH_3; 2′-CH_3↔2″-H_2; 2″-H_2↔1″-H_2; 1″-H_2↔2-OCH_3. HMBC (H→C) 2-OCH_3→2; 3-OH→2, 3, 4; 4-H→2, 3, 6; 5-H→1, 3, 6′; 2′-CH_3→1′, 2′, 3′; 3′-OH→2′, 3′, 4′; 4′-CH_3→3′, 4′, 5′; 5′-OCH_3→5′; 1″-H_2→1, 2, 6, 1′, 2″; 2″-H_2→1, 1′, 2′, 6′, 1″. EIMS (70 eV): *m*/*z* (%) = 300 (100, M^+), 285 (7), 253 (65), 150 (7), 115 (5). HREIMS *m*/*z* 300.1363 (calcd for $C_{18}H_{20}O_4$, 300.1362).

3.7. Stilbostemin G. 1-(3-Hydroxy-5-methoxy-4-methylphenyl)-2-(3-hydroxy-2- methoxyphenyl)-ethane (*4*)

UV λ^{MeOH} 279, 272, 216 nm. IR ν^{CHCl_3} cm^{-1} 3690 w, 3598 w, 3532 w, 3332 br, 2926 m, 2854 m, 1614 m, 1592 s, 1514 w, 1470 s, 1434 w, 1422 w, 1378 w, 1348 w, 1270 m, 1156 m, 1142 w, 1118 s, 1106 s, 1070 w, 1040 w, 998 m, 914 w, 838 w. ^1H and ^{13}C NMR see Table 1. NOESY: 2-OCH_3↔1″-H_2; 4-H↔5-H; 5-H↔6-H; 6-H↔1″-H_2 and 2″-H_2; 2′-H↔1↔-H_2 and 2″-H_2; 6′-H↔1″-H_2, 2″-H_2, and 5′-OCH_3. HMBC (H→C) 2-OCH_3 →2; 4-H→2, 3, 6; 5-H→1, 3, 6; 6-H→2, 4, 5, 1″; 2′-H→3′, 4′, 6′, 2″; 6′-H→2′, 4′, 5′, 2″; 4′-CH_3→3′, 4′, 5′; 5′-OCH_3→5′; 1″-H_2→1, 2, 6, 1′, 2″; 2″-H_2→1, 1′, 2′, 6′, 1″. EIMS (70 eV): *m*/*z* (%) = 288 (9, M^+), 257 (6), 151 (15), 137 (5), 86 (56), 84 (100), 59 (26). HREIMS *m*/*z* 288. 1356 (calcd for $C_{17}H_{20}O_4$, 288.1362).

3.8. Antifungal bioassay

Bioautography: 10 μg of pure compounds were applied on TLC plates (Merck, Si gel 60, 2.5 mm), separated with hexane–Et_2O (6:4), and sprayed with an aqueous spore suspension of *Cladosporium herbarum* containing malt bouillon (Merck) as nutrient. Plates were incubated in moist chambers for 3 days in the dark at room temp. White inhibition zones of different size indicated the degree of antifungal activity on the dark mycelial layer (Fig. 1).

Bioassays in microwells: Five microfungi, *Fusarium avenaceum* (Corda: Fr.) Sacc., *Alternaria citri* Ellis et Pierce emend. Bliss. et Fawcett, *Botrytis cinerea* Pers.: Fr., *Pyricularia grisea* (Cooke) Sacc., and *Cladosporium herbarum* (Pers.: Fr.) Link., were used for spore germination inhibition assay. Authentic samples were deposited at the culture collection of the Institute of Applied Microbiology, Agricultural University of Vienna (VIAM). Tests were carried out with a microplate reader SLT 400 ATC (SLT-Labinstruments Ges.m.b.H., Salzburg, Austria) as described in Table 2 and reported previously (Hadacek and Greger, 2000; Engelmeier et al., 2000; Pacher et al., 2002).

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