Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 69 (2008) 1185–1196
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Naphthoquinone spiroketal with allelochemical activity from the newly discovered endophytic fungus *Edenia gomezpompae*

Martha L. Macías-Rubalcava [a], Blanca E. Hernández-Bautista [a], Manuel Jiménez-Estrada [b], María C. González [d], Anthony E. Glenn [e], Richard T. Hanlin [f], Simón Hernández-Ortega [c], Aurora Saucedo-García [a], Jordi M. Muria-González [a], Ana Luisa Anaya [a,*]

[a] *Instituto de Ecología, Departamento de Ecología Funcional, Universidad Nacional Autónoma de México, Apartado Postal 70-275, Ciudad Universitaria, 04510 Mexico D.F., Mexico*
[b] *Instituto de Química, Departamento de Productos Naturales, Ciudad Universitaria, 04510 Mexico D.F., Mexico*
[c] *Laboratorio de Rayos X, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510 Mexico D.F., Mexico*
[d] *Instituto de Biología, Departamento de Botánica, Laboratorio de Micromicetos, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510 Mexico D.F., Mexico*
[e] *USDA, ARS, Toxicology and Mycotoxin Research Unit, Russell Research Center, Athens, GA 30604, USA*
[f] *University of Georgia, Museum of Natural History Annex, 4435 Atlanta Highway, Bogart, GA 30622, USA*

Received 18 April 2007; received in revised form 29 October 2007
Available online 29 January 2008

Abstract

Chemical investigation of the mycelium of *Edenia gomezpompae*, a newly discovered endophytic fungus isolated from the leaves of *Callicarpa acuminata* (Verbenaceae) collected from the ecological reserve El Eden, Quintana Roo, Mexico, resulted in the isolation of four naphthoquinone spiroketals, including three new compounds and palmarumycin CP_2 (**4**). We elucidated the structures of the metabolites by extensive NMR spectroscopy studies, including DEPT, COSY, NOESY, HSQC, HMBC, and chiroptical methods. The trivial names proposed for these compounds are preussomerin EG_1 (**1**), preussomerin EG_2 (**2**) and preussomerin EG_3 (**3**). In addition, the X-ray data for **4** were obtained. The bioactivity of the mycelial organic extracts and the pure compounds was tested against three endophytic fungi (*Colletotrichum* sp., *Phomopsis* sp., and *Guignardia manguifera*) isolated from the same plant species (*C. acuminata*, Verbenaceae) and against four economically important phytopathogenic microorganisms (two fungoid oomycetes, *Phythophtora capsici* and *Phythophtora parasitica*, and the fungi *Fusarium oxysporum* and *Alternaria solani*). Spiroketals **1–3** displayed significant growth inhibition against all the phytopathogens. IC_{50} values for the four phytopathogens were from 20 to 170 μg/ml. Palmarumycin CP_2 (**4**) was not bioactive against any of the fungi tested. Compound **1** showed the strongest bioactivity. The acetylated derivatives of preussomerin EG_1 (**1**), **1a** and **1b,** were obtained and their biological activity was tested on endophytes and phytopathogens. Preussomerin EG_1 **1**, **1a** and **1b** exhibited significant bioactivity against all microorganisms tested with the exception of *Alternaria solani*. This is the first report of allelochemicals with antifungal activity from the newly discovered endophytic fungus *E. gomezpompae*.

Keywords: *Edenia gomezpompae*; Endophytic fungi; *Callicarpa acuminata*; Verbenaceae; Allelochemicals; Naphthoquinone spiroketals; Preussomerin EG_1; Preussomerin EG_2; Preussomerin EG_3; Palmarumycin CP_2; Phytopathogenic fungi; Phytopathogenic fungoid oomycetes

1. Introduction

Endophytes are microbes that colonize living internal tissues of plants without causing any immediate, overt, negative effects or external symptoms. They are presumably ubiquitous in the plant kingdom. Colonization and

* Corresponding author. Tel.: +52 55 5622 9032; fax: +52 55 5622 9043.
E-mail address: alanaya@miranda.ecologia.unam.mx (A.L. Anaya).

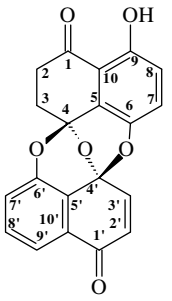
0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.12.006

propagation of endophytes may offer a significant benefit to their host plants by producing a plethora of metabolites that provide protection and survival value to the plants. These compounds could impact the broader ecological community as plant growth regulators, antimicrobials, antivirals, and insecticidals, or even mediate resistance to some types of abiotic stress. Endophytes also could be potential sources of novel natural products with agrochemical, pharmaceutical, and industrial potential (Strobel, 1996, 2006; Rodríguez, 1996; Strobel and Long, 1998; Bacon and White, 2000; Tan and Zou, 2001; Schulz et al., 2002; Strobel and Daisy, 2003; Strobel et al., 2004; Stone et al., 2004; Wiyakrutta et al., 2004; Gunatilaka, 2006).

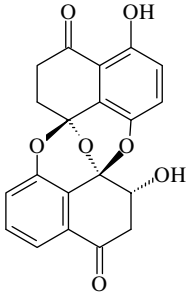
Among the large number of novel bioactive metabolites that are known from various fungi, the preussomerins and palmarumycins (deoxypreussomerins) belong to a relatively new and rare family of bioactive natural products based on a 1,8-dihydroxynaphthalene derived spiroketal unit linked to a second, oxidized naphthalene moiety (Weber and Gloer, 1991; Krohn et al., 2001; Hu et al., 2006; Jiao et al., 2006). These compounds exhibit an elaborate range of hydroxylation, oxidation, and unsaturation patterns. Preussomerins and deoxypreussomerins possess a wide range of biological properties, including antibacterial, antifungal, algicidal, herbicidal, antiplasmodial, and antitumor activities (Weber et al., 1990; Weber and Gloer, 1991; Krohn et al., 1994a,b, 2001; Soman et al., 1999; McDonald et al., 1999; Bode et al., 2000; Lazo et al., 2001; Wipf et al., 2001a; Seephonkai et al., 2002; Krohn, 2003; Prajoubklang et al., 2005). Some of these compounds have been identified as novel inhibitors of *ras*-farnesyltransferase (Singh et al., 1994; Vilella et al., 2000), DNA gyrase, topoisomerase II (Sakemi et al., 1995) and thioredoxin–thioredoxin reductase (Wipf et al., 2001b, 2004, 2005) and thus are of interest in terms of their potential in cancer chemotherapy.

As a part of a screening program directed towards the isolation of biologically active metabolites from plant species and endophytic fungi from the Ecological Reserve El Eden, Quintana Roo, Mexico (Anaya et al., 2003a,b, 2005; Macías-Rubalcava et al., 2007), we have investigated the allelochemical potential from the newly discovered endophytic fungus *Edenia gomezpompae*. This fungus belongs to a new species within the family Pleosporaceae (Order Pleosporales). Morphological, physiological, and molecular studies indicated that it belongs to a new genus and species (González et al., 2007).

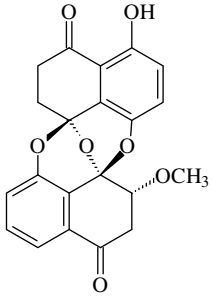
In the present paper, we report the isolation and structure elucidation of the major bioactive compounds from *E. gomezpompae*: the new naphthoquinone spiroketals preussomerin EG_1 (**1**), preussomerin EG_2 (**2**), and preussomerin EG_3 (**3**), and the known palmarumycin CP_2 or deoxypreussomerin B (**4**). In addition, we also present the results of antagonism tests of *E. gomezpompae* vs. phytopathogenic microorganisms and endophytic fungi, and the bioactivity of the four isolated and identified compounds against these microorganisms.



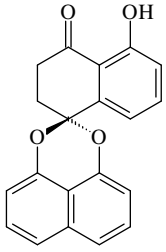
(**1**)



(**2**)



(**3**)



(**4**)

2. Results and discussion

The results presented come from the confrontation experiments to test for antagonisms. *E. gomezpompae* clearly inhibited the growth of the three other endophytic fungi: *Colletotrichum* sp., *Phomopsis* sp. and *Guignardia manguifera* that were isolated from *Callicarpa acuminata.* In the presence of *G. manguifera, Edenia* shows a tendency to change its morphology, and *G. manguifera* produces a darker color (Fig. 1). Four economically important phytopathogens: *Phythophtora parasitica* and *Phythophtora capsici* (Oomycota), and *Fusarium oxysporum* and *Alternaria solani* (Eumycota), also were inhibited by the presence of *E. gompezpompae,* particularly the two species of *Phythophtora* (Fig. 2).

E. gomezpompae was cultivated using fermentation in potato dextrose liquid medium (PD). Organic extracts of both the culture medium and mycelium showed significant activity against the endophytic fungi *Phomopsis* sp. and *Colletotrichum* sp. (Fig. 3a), but the culture medium extract had no effect against *G. manguifera* growth; however, this fungus was significantly inhibited by the mycelial extract of *E. gomezpompae*. The culture medium extract significantly inhibited the radial growth of the four phytopathogenic species (Fig. 3b). *P. capsici* and *P. parasitica* were inhibited 100% by the culture medium extract. The mycelial extract also significantly inhibited the growth of these two species but did not significantly inhibit *A. solani* or *F. oxysporum*.

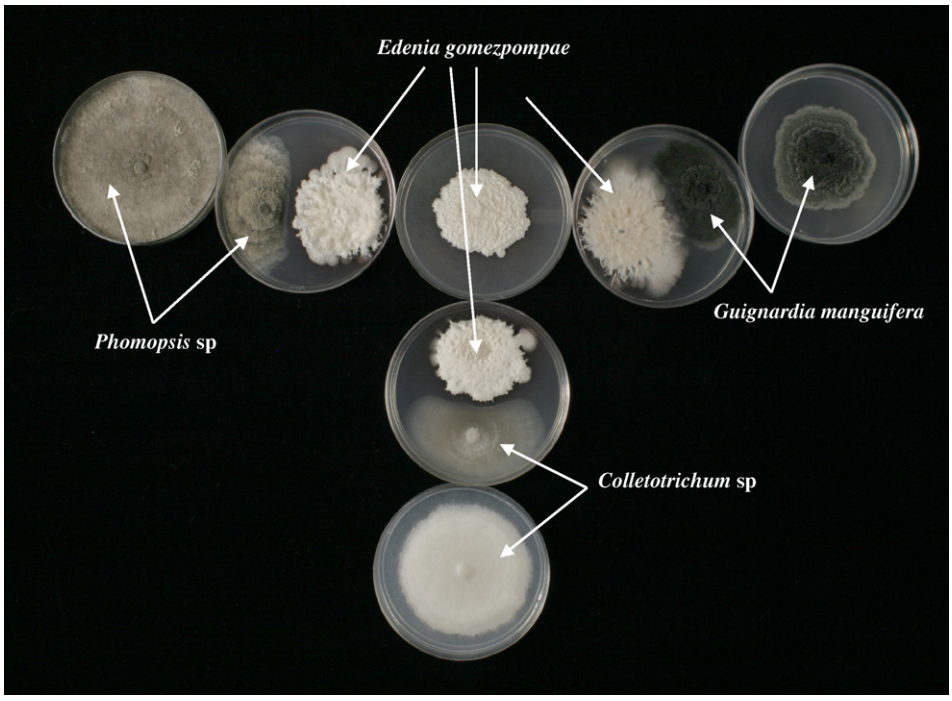


Fig. 1. Antagonism bioassays with *Edenia gomezpompae* and other endophytic fungi.

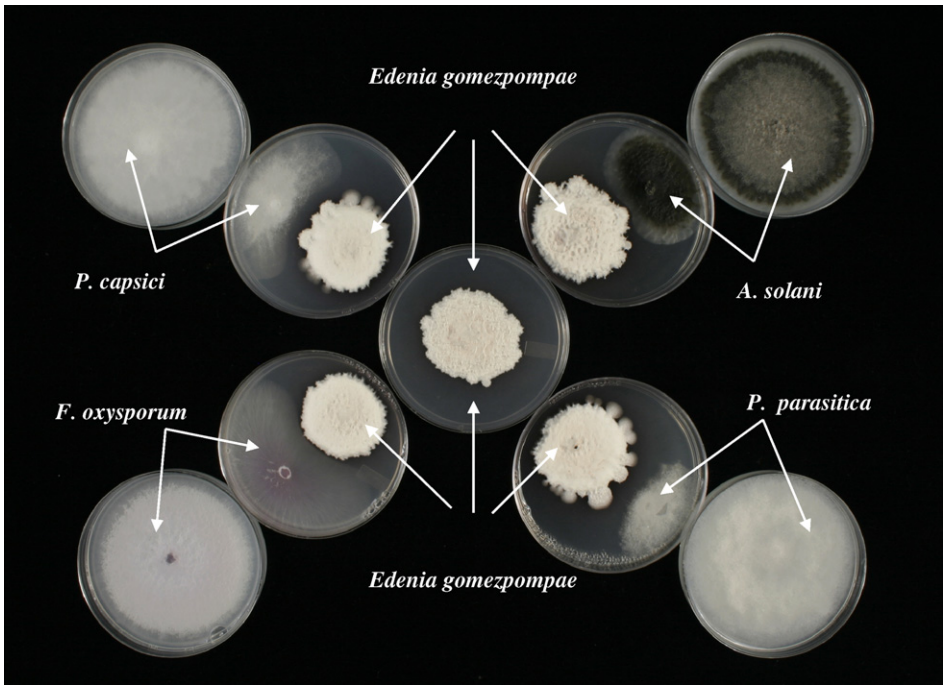


Fig. 2. Antagonism bioassays with *Edenia gomezpompae* and some phytopathogenic microorganisms.

The culture medium extract was more active against *P. capsici* and *P. parasitica* ($IC_{50} = 47.8$ and 125.9 μg/ml, respectively), while the mycelial extract was more active against *P. parasitica* and *Phomopsis* ($IC_{50} = 169.8$ and 313.5 μg/ml, respectively). The IC_{50} value of culture medium for *P. capsici* was similar to the IC_{50} value of the fungicide Captan® (Table 1). Considering these results and the extract yields obtained from the mycelium and culture medium (14.25 g and 830 mg, respectively), we selected the mycelial extract for the bioassay-guided fractionation.

Bioassay-guided fractionation of the mycelial extract from *E. gomezpompae* led to the isolation of four naphthoquinone spiroketal derivatives. These compounds included three new preussomerins that were characterized by

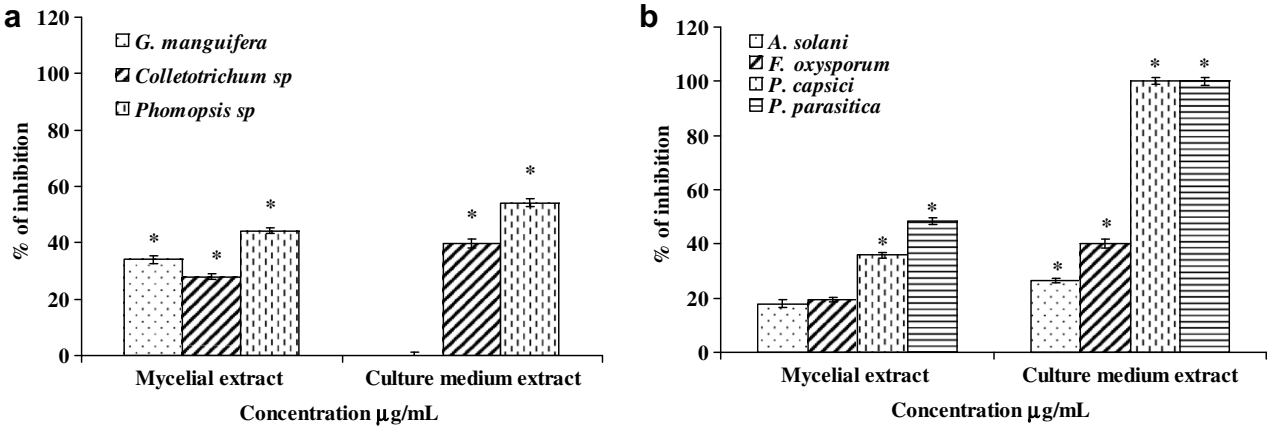


Fig. 3. Radial growth-inhibitory activity of the culture medium and mycelial extracts (250 µg/ml) from endophytic fungus *Edenia gomezpompae* against (a) endophytic fungi isolated from *Callicarpa acuminata* and (b) phytopathogenic microorganisms with economic importance. Vertical bars represent SD, $N = 4$; $^{*}P < 0.05$.

Table 1
Bioactivity of the culture medium and mycelial extracts from endophytic fungus *Edenia gomezpompae* on diameter growth (IC_{50}, µg/ml) of phytopathogens with economic importance and known endophytic fungi isolated from *Callicarpa acuminata*

Test organisms	Culture medium extract	Mycelial extract	Captan
Phytopathogens			
Alternaria solani[a]	>500	>500	22.9
Fusarium oxysporum[a]	380.2	>500	28.4
Phythophtora capsici[b]	47.8	458.3	46.1
Phythophtora parasitica[b]	125.9	169.8	12.1
Endophytic fungus			
Colletotrichum sp.[b]	373.8	>500	40.1
Phomopsis sp.[b]	216.3	313.5	4.7
Guignardia manguifera[c]	>500	>500	51.9

IC_{50} (the effective dose for 50% diameter growth reduction).
[a] Results after 4 days of incubation.
[b] Results after 3 days of incubation.
[c] Results after 10 days of incubation.

spectroscopic and chiroptical methods. The trivial names proposed for the new spiroketals are preussomerin EG_1 (**1**), preussomerin EG_2 (**2**), and preussomerin EG_3 (**3**). In addition, the known palmarumycin CP_2 (deoxypreussomerin B) (**4**) was obtained.

Analysis of preussomerin EG_1 (**1**) by HREIMS and ^{13}C NMR spectroscopy indicated that it has the molecular formula $C_{20}H_{12}O_6$ (15 degrees of unsaturation). The infrared spectrum of **1** showed absorption bands for a conjugated carbonyl at 1642 cm^{-1}, an α,β-unsaturated carbonyl group at 1679 cm^{-1} and an hydroxyl group at 3078 cm^{-1}. The ^1H NMR spectrum (Table 2) contained resonances indicating the presence of 1,2,3-trisubstituted and 1,2,3,4-tetrasubstituted aromatic rings. These resonances correspond to two sets of *o*-related aromatic protons at δ_H 6.61 (*d* $J = 9.0$ Hz, H-8) and 7.01 (*d* $J = 9.0$ Hz, H-7) ppm and δ_H 6.56 (*d* $J = 9.5$ Hz, H-2′) and 7.71 (*d* $J = 9.5$ Hz, H-3′) ppm. These last proton resonances (δ_H 6.56 and 7.71) are typical of a *Z*-olefin fragment that could belong to an α,β-unsaturated carbonyl group. In addition, these spectra exhibited the presence of a hydrogen atom of a hydroxyl group at δ_H 11.71 (OH-9) ppm, which is chelated with a carbonyl group and slowly exchanges with deuterium upon shaking with D_2O. Two methylene groups between δ_H 2.53 and 3.35 ($H_{a,b}$-2 and $H_{a,b}$-3) ppm, and three aromatic protons between δ_H 7.03-7.59 (H-7′, H-8′ and H-9′) ppm, were assigned based on the connections observed in the ^1H–^1H COSY and ^1H–^1H NOESY experiment (Table 3). The ^{13}C NMR spectrum (Table 2) of **1** exhibited 20 carbon signals which were assigned with the help of an HSQC experiment. Two of the quaternary carbon signals appeared at δ_C 89.6 (C-4′) and 93.7 (C-4) ppm, that are characteristic of the spiroketal carbons of the preussomerins (Weber et al., 1990; Weber and Gloer, 1991; Singh et al., 1994; Krohn et al., 2001; Quesada et al., 2004). The other carbons were identified as two conjugated carbonyls appearing at δ_C 184.07 (C-1′) and 201.9 (C-1) ppm and seven olefinic or aromatic methines, one of which appeared at δ_C 141.2 (C-3′) ppm and was assigned to a carbon β to the carbonyl group. Two carbon signals at δ_C 33.7 and 32.6 ppm correspond to the methylene groups C-2 and C-3, respectively. The remaining seven carbons are quaternary and were assigned as shown in Table 2. The structure of **1** was also supported by the HMBC experiment of the molecule (Fig. 4).

HREIMS and ^{13}C NMR analysis of preussomerin EG_2 (**2**) gave a molecular formula of $C_{20}H_{14}O_7$ (14 degrees of unsaturation). The ^1H NMR spectrum (Table 2) again contained resonances for the characteristic trisubstituted and tetrasubstituted aromatic rings. Comparison of the ^1H NMR spectrum of **2** with that of preussomerin EG_1 (**1**) indicated absence of the C-2′, C-3′ double bond, the presence of a hydroxyl group at H-3′/C-3′ (δ_H/δ_C 2.38/70.3 ppm), and an apparent triplet of an oxygenated methine proton at δ_H 4.71 (1H, pseudo-t *dd*, $J = 3.0$, 3.0 Hz, H-3′) ppm as well as two self-coupled aliphatic proton signals at δ_H 3.02 ($J = 3.0$, 18.3 Hz; H-2′a) and 3.38 ($J = 3.0$, 18.3 Hz, H-2′b) ppm, which in turn were coupled

Table 2
^{1}H and ^{13}C NMR spectroscopic data for preussomerins EG_1, EG_2 and EG_3 isolated from *Edenia gomezpompae*[a,b]

Position	Preussomerin EG_1 (1)		Preussomerin EG_2 (2)		Preussomerin EG_3 (3)	
	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
1	201.9	–	202.0	–	202.2	–
2a	33.7	2.90 (1H, *ddd* 19.0, 5.5, 2.0)	33.7	2.91 (1H, *ddd* 18.8, 13.5, 5.5)	33.8	2.90 (1H, *ddd* 18.6, 5.5, 2.0)
2b		3.35 (1H, *ddd* 19.0, 13.5, 5.5)		3.37 (1H, *ddd* 18.8, 5.5, 2.0)		3.37 (1H, *ddd* 18.6, 13.5, 5.5)
3a	32.6	2.53 (1H, *ddd* 13.5, 13.5, 5.5)	32.7	2.50 (1H, *ddd* 13.5, 13.5, 5.5)	32.8	2.50 (1H, *ddd* 13.5, 13.5, 5.5)
3b		2.74 (1H, *ddd* 13.5, 5.5, 2.0)		2.77 (1H, *ddd* 13.5, 5.5, 2.0)		2.80 (1H, *ddd* 13.5, 5.5, 2.0)
4	93.7	–	93.4	–	93.2	–
5	119.5	–	120.1	–	120.3	–
6	141.4	–	140.9	–	140.9	–
7	126.2	7.01 (1H, *d* 9.0)	126.0	7.04 (1H, *d* 9.0)	126.0	7.02 *d* (9.0)
8	120.7	6.91 (1H, *d* 9.0)	120.9	6.94 (1H, *d* 9.0)	120.7	6.92 *d* (9.0)
9	157.3	–	157.5	–	157.3	–
10	112.6	–	113.0	–	113.0	–
1′	184.1	–	193.6	–	193.7	–
2a′	133.3	6.56 (1H, *d* 9.5)	41.3	3.02 (1H, *dd* 3.0, 18.3)	40.4	3.08 (1H, *dd* 3.0, 18.0)
2b′				3.38 (1H, *dd* 3.0, 18.3)		3.39 (1H, *dd* 3.0, 18.0)
3′	141.2	7.17 (1H, *d* 9.5)	70.3	4.71 (1H, pseudo *t dd* 3.0, 3.0)	79.3	4.27 (1H, pseudo t *dd* 3.0, 3.0)
4′	89.6	–	94.0	–	94.4	–
5′	120.6	–	119.3	–	119.9	–
6′	149.5	–	150.8	–	150.6	–
7′	120.4	7.59 (1H, *dd* 7.5, 1.0)	121.5	7.09 (1H, *dd* 8.0,1.0)	121.6	7.08 (1H, *dd* 8.0,1.0)
8′	130.8	7.38 (1H, *dd* 8.0, 7.5)	131.1	7.41 (1H, *dd* 8.0, 8.0)	130.9	7.38 (1H, *dd* 8.0, 8.0)
9′	120.6	7.03 (1H, *dd* 8.0, 1.0)	120.4	7.66 (1H, *dd* 8.0, 1.0)	120.3	7.63 (1H, *dd* 8.0, 1.0)
10′	130.4	–	130.8	–	130.9	–
OH-3′	–	–	–	2.38 (1H, *s*a)	–	
OH-9	–	11.71 (1H, *s*)	–	11.71 (1H, *s*)	–	11.71 (1H, *s*)
CH_3O-3′	–	–	–		59.2	3.51(3H, *s*)

[a] ^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$ at 500 and 125 MHz, respectively; TMS was used as internal standard; chemical shifts are shown in the δ scale with J values (Hz) in parentheses.
[b] Assignments are based on DEPT, HMBC, HSQC, ^{1}H–^{1}H COSY and ^{1}H–^{1}H NOESY experiments, and chemical shift values.

to the methine proton and correlated with a hydroxyl group in NOESY spectra (Table 3).

The α configuration was assigned to the stereogenic center at C-3′ based on the following observation. The small coupling constant of 3.0 and 3.0 Hz between H-3′ and two methylene protons H-2a′ and H-2b′ in the ^{1}H NMR spectrum of preussomerin E_2 suggests that H-3′ must be placed in the *pseudo*-equatorial position and thus the hydroxyl group must be placed in a *pseudo*-axial position of the pseudochair ring conformation. (Weber and Gloer, 1991; Singh et al., 1994; Soman et al., 1999; Seephonkai et al., 2002). This observation was strongly supported by application of chiroptical methods for the assignment of the absolute configuration. This method was previously applied successfully to elucidate the absolute configurations of several palmarumycins and preussomerins. The preussomerins are ideal substrates for this kind of determination since they are conformationally very rigid molecules, fixed by their bisspiro structure (Bringmann et al., 1997; Krohn et al., 1997a,b, 2001). The CD spectrum of preussomerin EG_2 (**2**) showed a negative $n \rightarrow \pi^*$ Cotton effect around 334 and 218 nm, supporting the depicted 3′*R* absolute configuration (Krohn et al., 2001).

Preussomerin EG_3 (**3**) gave a molecular formula $C_{21}H_{16}O_7$ (14 degrees of unsaturation) as deduced from HREIMS in agreement with the number of resonances for carbon and hydrogen atoms, detected in the NMR spectra. In fact, the ^{1}H NMR and ^{13}C NMR spectrum of preussomerin EG_3 (**3**) are almost identical to that of preussomerin EG_2 (**2**), except for the absence of free hydroxyl group at C-3′ and the presence of a methoxy group at δ_H 3.51 ppm. Analysis of HMBC data, chemical shifts, and ^{1}H NMR J values verified the structure of **3** as a 3′-*O*-methyl analogue of preussomerin EG_2 (**2**). The data of the ^{1}H and ^{13}C NMR spectra, together with the correlations resulting from the ^{1}H–^{1}H NOESY and HMBC experiments, are listed in Tables 2 and 3. Once more, the absolute stereochemistry at C-3′ was assigned based on the negative Cotton effect at 334 nm in the CD spectrum. Consequently, the configuration at C-3′ was established as *R* (Krohn et al., 2001).

The new preussomerins **1**, **2**, and **3** differ from other known natural preussomerins by the missing epoxide in position 2 and 3 (Singh et al., 1994; Soman et al., 1999; Seephonkai et al., 2002). The spectral properties of the known palmarumycin CP_2 (**4**), including IR, ^{1}H NMR, and ^{13}C NMR spectroscopic data, were identical to those previously described in the literature (Krohn et al., 1994a,b; Singh et al., 1994; Ragot et al., 1999; Barrett et al., 2002). X-ray data of palmarumycin CP_2 (**4**) were not published previously; therefore, we are describing X-ray data of this compound in the present study. The structure and

Table 3
HMBC and NOESY spectroscopic data for Preussomerins EG_1, EG_2 and EG_3 isolated from *Edenia gomezpompae*[a]

Position	Preussomerin EG_1 (1)		Preussomerin EG_2 (2)		Preussomerin EG_3 (3)	
	HMBC (C → H)	NOESY	HMBC (C → H)	NOESY	HMBC (C→ H)	NOESY
1	2a, 2b, 3a, 3b, 8	–	2a, 2b, 3a, 3b, 8	–	2a, 2b, 3a, 3b, 8	–
2	3a, 3b	2b, 3a, 3b 2a, 3a, 3b	3a, 3b	2b, 3a, 3b 2a, 3a, 3b	3a, 3b	2b, 3a, 3b 2a, 3a, 3b
3	2a, 2b	2a, 2b, 3b 2a, 2b, 3a	2a, 2b	2a, 2b, 3b 2a, 2b, 3a	2a, 2b	2a, 2b, 3b 2a, 2b, 3a
4	2a, 2b, 3a, 3b, 7	–	2a, 2b, 3a, 3b, 7	–	2a, 2b, 3a, 3b, 7	–
5	3a, 3b, 7, 8	–	3a, 3b, 7, 8	–	3b, 7, 8	–
6	3b, 7, 8	–	3b, 7, 8	–	3b, 7, 8	–
7	OH-9	8, OH-9	8, OH-9	8	8, OH-9	8
8	7, OH-9	7, OH-9	7, OH-9	7, OH-9	7, OH-9	7, OH-9
9	7, 8, OH-9	–	7, 8, OH-9	–	7, 8	–
10	2a, 7, 8, O H-9	–	2a, 7, 8, OH-9	–	2a, 7, 8, O H-9	–
1′	2′, 3′, 7′, 8′	–	2a′, 2b′, 3′, 8′, 9′	–	2a′, 2b′, 3′, 8′, 9’	–
2′	–	3′	–	2b′, 3′ 2a′, 3′	–	2b′, 3′ 2a′, 3′
3′	–	2′	2a′, 2b′,	2a′, 2b′, OH-3	2a′, 2b′, CH_3O-3′	2a′, 2b′, CH_3O-3′
4′	2′, 3′, 7′, 9′	–	2a′, 3′, 7′, 9′	–	2a′, 3′, 7′, 9′	–
5′	3′, 7′, 8′, 9′	–	3′, 7′, 8′, 9′	–	3′, 7′, 8′, 9′	–
6′	3a, 3′, 7′, 8′, 9′	–	3a,7′, 8′, 9′	–	3a,7′, 8′, 9′	–
7′	8′, 9′	8′, 9′	8′, 9′	8′	8′, 9′	8′
8′	7′ 9′	7′, 9′	7′ 9′	7′, 9′	7′, 9′	7′, 9′
9′	2′, 7′, 8′	8′, 7′	7′ 8′	8′, 7′	7′, 8′, OH-9	8′, 7′
10′	2′, 8′ 9′	–	2a′, 7′, 8′ 9′	–	2a′, 7′, 8′ 9′	–
OH-3′	–	–	–	2a′, 2b′, 3′	–	–
OH-9	–	8	–	8	–	8
CH_3O-3′	–	–	–	–	2b′, CH_3O-3′	–

[a] HMBC and NOESY spectra were acquired in $CDCl_3$ at 500 MHz; TMS was used as internal standard; chemical shifts are shown in the δ scale.

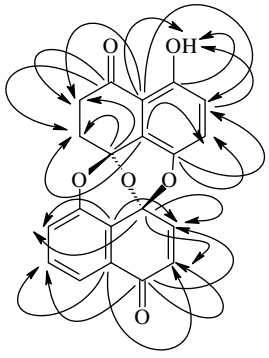
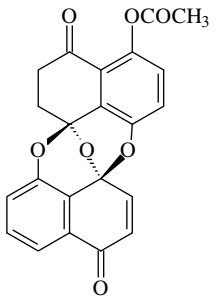


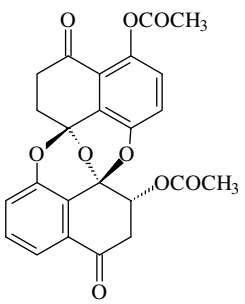
Fig. 4. Key ^{13}C–^{1}H long range correlations observed in HMBC spectra for preussomerin EG_1 (**1**).

relative configuration were confirmed by single crystal diffraction. An ORTEP drawing is displayed in Fig. 5.

The natural compounds **1**–**4** and their derivatives (**1a** and **1b**) were tested for their bioactivity against the two phytopathogenic fungi (*F. oxysporum* and *A. solani*), and the phytopathogenic fungoid oomycetes *P. parasitica* and *P. capsici*. Compound **1** and its derivatives were tested on the three endophytic fungi (*Colletotrichum* sp., *Phomopsis* sp., and *G. manguifera*). The results of these bioassays are shown in Figs. 6 and 7. Preussomerins **1**–**3** exhibited significant inhibitory activity against all phytopathogenic fungi at 100 μg/ml. In contrast, palmarumycin CP_2 (**4**) did not show significant antifungal activity. It is possible to see a continuum between the potency for growth inhibition for compounds **1–4** (Fig. 6). Preussomerin EG_1 (**1**) exhibited the strongest bioactivity causing complete growth inhibition of *P. parasitica*, *P. capsici* and *F. oxysporum*. *A. solani* was not inhibited by **1;** however, compounds **2** and **3** inhibited its growth significantly (Fig. 6). The IC_{50} values of the isolated compounds on the radial growth of phytopathogenic test microorganisms show that compounds **1–3** reduced the diameter growth of all target species in a concentration-dependent manner. Preussomerin EG_1 (**1**) was shown to be more active than the commercial fungicide Captan® on the growth of *P. capsici* (Table 4).



(**1a**)



(**1b**)

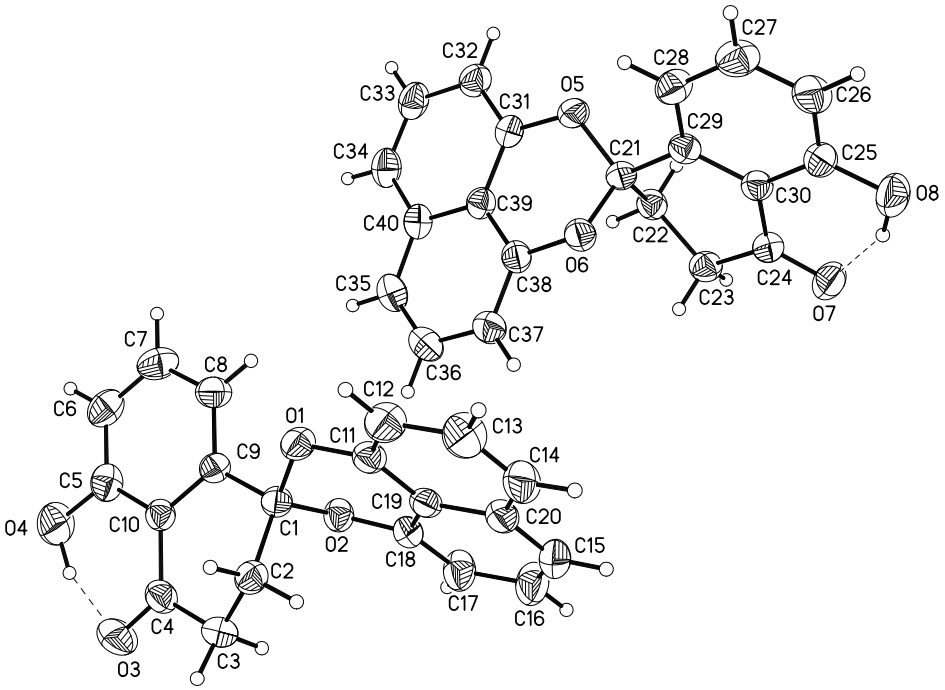


Fig. 5. ORTEP drawing of palmarumycin CP_2 (**4**).

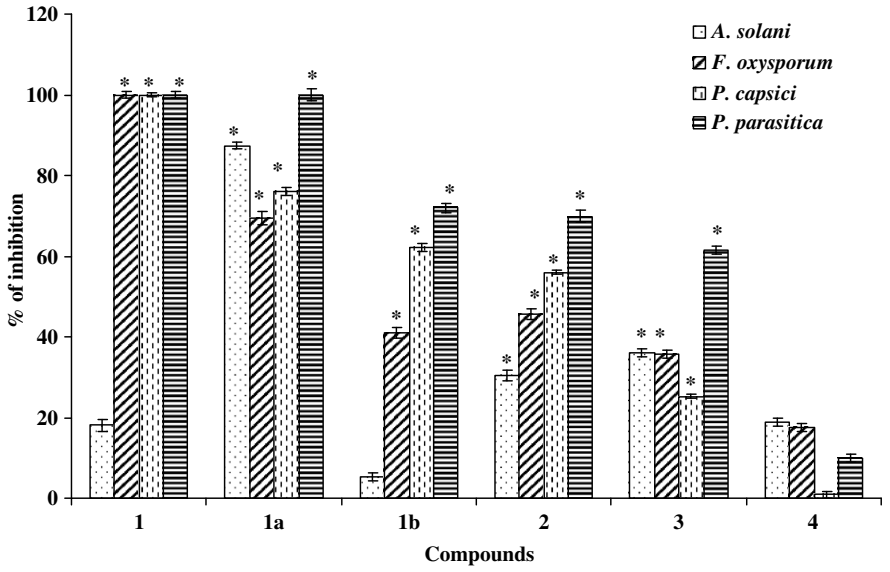


Fig. 6. Diameter growth-inhibitory activity of the isolated compounds (100 μg/ml) from endophytic fungus *Edenia gomezpompae* against phytopathogens *A. solani, F. oxysporum*, *P. parasitica* and *P. capsici*. Vertical bars represent SD, $N = 4$; $^{*}P < 0.05$.

Table 4
Bioactivity of the isolated compounds from endophytic fungus *Edenia gomezpompae* on the diameter growth (IC_{50}, M) of *Alternaria solani, Fusarium oxysporum*, *Phythophtora parasitica* and *Phythophtora capsici*

Compound	*A. solani*	*F. oxysporum*	*P. parasitica*	*P. capsici*
1	$>5.75 \times 10^{-4}$ M	1.45×10^{-4} M	57.71×10^{-5} M	5.61×10^{-5} M
2	$>5.46 \times 10^{-4}$ M	3.11×10^{-4} M	1.58×10^{-4} M	2.14×10^{-4} M
3	$>5.26 \times 10^{-4}$ M	4.47×10^{-4} M	1.95×10^{-4} M	$>5.26 \times 10^{-4}$ M
4	$>6.29 \times 10^{-4}$ M	$>6.29 \times 10^{-4}$ M	$>6.29 \times 10^{-4}$ M	$>6.29 \times 10^{-4}$ M
Captan	7.62×10^{-5} M	4.46×10^{-5} M	4.04×10^{-5} M	1.53×10^{-4} M

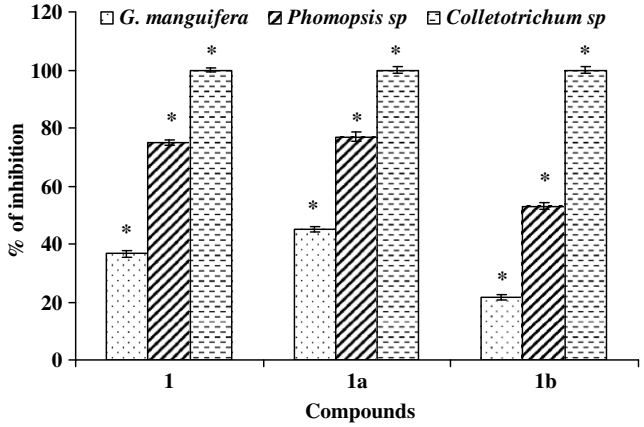


Fig. 7. Radial growth-inhibitory activity of preussomerin EG_1 (**1**) and semi-synthetic derivatives **1a** and **1b** (100 μg/ml) against the endophytic fungi *Colletotrichum* sp., *Phomopsis* sp., and *Guignardia manguifera* isolated from *C. acuminata*. Vertical bars represent SD, $N = 4$; $^{*}P < 0.05$.

Considering that preussomerin EG_1 (**1**) was the most abundant natural product isolated from the mycelial extract (3.4 mg/l of culture medium) and it displayed the highest antifungal activity on almost all the fungi tested, we semi-synthesized two acetylated derivatives of preussomerin EG_1 (**1**). Treatment of preussomerin EG_1 (**1**) with Ac_2O/pyridine afforded a monoacetylpreussomerin (**1a**) and a diacetylated product (**1b**). Diacetylpreussomerin **1b** was obtained by addition of acetate in a Michael addition to the enone system of **1**. Monoacetylpreussomerin (**1a**) significantly inhibited the four phytopathogens (Fig. 6). Diacetylpreussomerin EG_1 (**1b**) significantly inhibited *F. oxysporum*, *P. capsici*, and *P. parasitica* but did not inhibit *A. solani.* Results of bioassays of compound **1** and its two derivatives against endophytic fungi (Fig. 7) showed that the three compounds (100 μg/ml) significantly inhibited the mycelial growth of the three endophytic fungi, however *Colletotrichum* sp. was the most inhibited (100%).

3. Concluding remarks

Considering the results, some structure–activity relationships within this class of antifungal agents were established. When comparing the chemical structure of the preussomerin EG_1 (**1**) with **2** and **3** and the acetylated derivatives **1a** and **1b**, the presence of the C-2′, C-3′ double bond is evident in the most bioactive compounds (**1** and **1a**). The absence of this double bond in compounds **2, 3,** and **1b** possibly determined a less inhibitory effect. This observation indicates that the presence of a hydroxyl (**2**), methoxy (**3**), and acetyl groups (**1b**) at C-3′ possibly diminished the bioactivity. On the other hand, a positive effect of a hydroxyl group at C-9 was evident by comparing the activities of **1** and **1a.** Both compounds differ by the presence of the OH-9 or $OCOCH_3$-9, respectively. In conclusion, the structure–activity relationship reveals that the hydroxyl group at C-9 and the presence of the C-2′, C-3′ double bond are possibly responsible for the higher bioactivity of **1**.

Little biology is known regarding endophytes in tropical forest trees, where their abundance and diversity are thought to be greatest. Arnold et al. (2001) explored the occurrence of endophytes in a broad diversity of woody, angiospermous taxa in a lowland, moist tropical forest in central Panama. Fungal endophytes were found in every plant species examined and appear to be important, but largely immeasurable components of fungal biodiversity. Schulz et al. (1999) and Schulz and Boyle (2005) mentioned that there are no neutral interactions, but rather that endophyte-host interactions involve a balance of antagonisms, irrespective of the plant organ infected. There is always at least a degree of virulence on the part of the fungus, which enables infection of the host, whereas defense by the plant host limits colonization by fungal invaders and development of disease. It is also hypothesized that the endophytes, in contrast to known phytopathogens, generally have far greater phenotypic plasticity with regard to infection, local but also extensive colonization, latency, virulence, pathogenicity and (or) saprophytism. This phenotypic plasticity is suggested to be a motor of evolution.

The present study contributes to the knowledge of endophytic fungi of *C. acuminata*, a Verbenaceous shrub species from the low and medium tropical forest of Yucatan peninsula. *E. gomezpompae* has never been chemically explored, and this is the first report on bioactive compounds from this fungus. Results on bioactivity of the spiroketal type compounds that it produces lead us to hypothesize that they could be involved in the antagonic balance between plant and fungi defenses.

Further studies on endophytic fungi in plant communities of this zone will contribute valuable information on the endophytic fungal species associated with rich plant diversity in natural conditions. New species of fungi are likely to be discovered, and bioprospecting studies on these fungi may allow us to discover new natural products or allelochemicals with agrochemical and pharmacological potential.

4. Experimental

4.1. General experimental procedures

Melting points were measured in a Fisher–Johns apparatus and are uncorrected. The IR spectra were obtained using KBr disks on a Perkin–Elmer 599-B spectrophotometer. UV spectra were obtained on a Shimadzu 160 UV spectrometer in MeOH solution. CD spectra were performed on a JASCO 720 spectropolarimeter at 25 °C in MeOH solution. Optical rotations were taken on a JASCO DIP 360 polarimeter. NMR spectra including COSY spectra, NOESY, HMBC and HMQC experiments were recorded on a Bruker DMX500, in $CDCl_3$, either at 500

(^{1}H) or 125 (^{13}C) MHz, using tetramethylsilane (TMS) as an internal standard. EI mass spectra were performed using a Hewlet-Packard 5890 mass spectrometer. X-ray analysis of compound **4** was accomplished on a Siemens P4/PC diffractometer equipped with graphite-monochromated radiation. Open column chromatography (CC): silica gel 60 (70–230 mesh, Merck). Analytical and preparative TLC were performed on pre-coated silica gel 60 F_{254} plates (Merck).

4.2. Fungal material

The newly discovered fungus *E. gomezpompae* was isolated from surface sterilized (Rodríguez, 1994) leaves of *C. acuminata* (Verbenaceae) collected at the Ecological Reserve El Eden, Quintana Roo, Mexico in May 2002. A specimen of the plant was deposited in the University of California, Riverside Herbarium (2641. L.M. Ortega-Torres). Cultures of the fungus have been preserved lyophilized in N_2. Subcultures were obtained in several culture media, such as PDA, V8 agar, and others. Dried cultures of this fungus have been deposited in the J.H. Miller Mycological Herbarium (GAM) of the University of Georgia and in the fungi collection of Herbario Nacional de México (MEXU), UNAM, under the accession numbers GAM 16175 and MEXU 25346, respectively.

4.3. Antagonism bioassays with E. gomezpompae against endophytic fungi and some phytopathogenic microorganisms

Antagonism bioassays were performed against three endophytic fungi that were also isolated from *C. acuminata* (*Colletotrichum* sp., *Phomopsis* sp., and *G. manguifera*) and four economically important phytopathogenic microorganisms, two phytopathogenic fungi (Eumycota): *Fusarium oxysporum* and *Alternaria solani,* and two phytopathogenic fungus-like organisms (Oomycota): *Phythophtora parasitica* and *Phythophtora capsici*. Nowadays, these fungoids oomycetes are classified within the phylum Heterokontophyta (class Oomycota) which are primarily algae). The bioassays were performed in 9 cm Petri dishes with PDA medium. Because *E. gomezpompae* grows very slowly, inoculum (5 mm^3 agar plug) was placed one side of the dish and grown for 15 days at 28 °C and a photoperiod of 12:12 with natural light before the antagonism test. Inoculum of the respective test fungus then was added to the other side of the dish. The dish was sealed with Parafilm® and incubation was continued under the same conditions. Four replicate dishes were used for each antagonism bioassay. Controls consisted of each fungus growing individually under the same conditions. Results of the antagonism tests were observed at different times of incubation, depending on the growth rate of the test microorganism; *A. solani* and *F. oxysporum* after 4 days of incubation; *P. capsici*, *P. parasitica*, *Colletotrichum* sp. and *Phomopsis* sp. after 38 days of incubation; and *G. manguifera* after 10 days of incubation. The development of both microorganisms growing together was compared with the respective controls.

4.4. Cultivation

Fresh mycelium grown on potato-dextrose agar (PDA) (Hanlin and Ulloa, 1988) medium at 28 °C for 15 days was inoculated into one 1000 ml Erlenmeyer flasks containing 500 ml potato-dextrose (PD) broth. After 15 days of incubation at 28 °C on rotary shaker at 200 rpm, this culture was transferred as inoculum into fermentation equipment. Fermentation was done on a 30 l scale (PD medium) in three M19-1400 fermenters, New Brunswick scientific Co., Edison, NJ, USA). The fungus was grown at 28 °C for 3 weeks with stirring at 200 rpm and an aeration rate of 5 l/min.

4.5. Extraction and isolation of allelochemicals from E. gomezpompae

At the end of fermentation, the culture medium was separated from the mycelium by filtration. The culture medium (27 l) was concentrated at room temperature and high vacuum to 3 l and was extracted three times with 3 l of CH_2Cl_2 and then three times with 3 l of EtOAc. Both organic phases were combined and filtered over anhydrous Na_2SO_4, and concentrated *in vacuo* to give a reddish solid (830 mg). The mycelium was extracted the same way to yield 14.25 g of a reddish solid.

The mycelial extract was subjected to silica gel CC (120 g) eluting with a gradient of *n*-hexane–CH_2Cl_2–MeOH mixtures of increasing polarity. From this chromatography, 175 fractions (100 ml each) were obtained. Thirteen fractions, F-I to F-XIII, were pooled according to TLC analysis. Bioactivity in the antifungal bioassay showed nine active pools: F-IV (2758 mg), F-V (239 mg), F-VI (72 mg), F-VII (86 mg), F-VIII (204 mg), F-IX (1256 mg), F-X (135 mg), F-XII (278 mg), and F-XIII (748 mg).

Active fraction F-IV, eluted with hexane–CH_2Cl_2 (5:5), was resolved by preparative TLC (*n*-hexane–CH_2Cl_2; 2:8) to yield palmarumycin CP_2 (**4**) (25.4 mg) (Krohn et al., 1994a,b; Singh et al., 1994; Ragot et al., 1999; Barrett et al., 2002). From primary fraction V (eluted with *n*-hexane–CH_2Cl_2 (3:7), spontaneously crystallized preussomerin EG_1 (**1**) (24.1 mg). Primary fraction VII (eluted with CH_2Cl_2), was resolved by successive preparative TLC ($CH_2Cl_2 \times 3$) to yield preussomerin EG_1 (**1**) (43.9 mg). Extensive TLC (CH_2Cl_2–MeOH; 99.05:0.5) of fraction F-VIII eluted with CH_2Cl_2–MeOH (99:1) yielded palmarumycin EG_3 (**3**) (26.4 mg). Bioactive fraction F-IX, eluted with CH_2Cl_2–MeOH (97:3) was applied to a Si gel (60 g) column eluted with a gradient of *n*-hexane–CH_2Cl_2 (5:5 → 0:10) and CH_2Cl_2–MeOH (9.9:0.1 → 9:1). Eight secondary fractions were obtained (F-IX1–F-IX8). According to the bioassay, the bioactivity was concentrated in secondary fraction F-IX6. Fraction F-IX6 (426 mg), eluted with CH_2Cl_2–MeOH (99:1) was further resolved on another Si gel (40 g) column using the same elution system as for fraction F-IX to yield seven tertiary fractions (FIX6-1–FIX6-7). The activity was found in

tertiary fraction F-IX6-4 (77.5 mg), eluted with CH_2Cl_2. Finally, extensive TLC (CH_2Cl_2–MeOH; 98:2 × 2) of fraction F-IX6-4 yielded preussomerin EG_2 (**2**) (28.3 mg).

4.6. Bioassays with phytopathogenic and endophytic fungi

Organic extracts, fractions, and isolated compounds were tested for inhibitory effects on the growth of the seven test microorganisms. The extracts (culture and mycelium) were evaluated at 75, 125, 250 and 500 μg/ml by dilution in agar. Each treatment was added to sterile PDA (40 °C) before solidification of the agar in 6-cm Petri dishes. Fractions were evaluated at 250 μg/ml and pure compounds **1–4** and **1a** and **1b** were evaluated using four different concentrations depending on the test microorganism. The commercial fungicide Captan® (*cis*-*N*-[(trichloromethyl)thio]4-cyclo-hexene-1,2-dicarboximide) was used as a positive control. The bioassays were performed by following a completely random design with four replicates. Inoculated plates were incubated in darkness at 27 °C. The effects of treatments were determined by measuring the diameter growth of the mycelium after 3, 4 and 10 days of incubation depending on the growth speed of the test microorganism. Two perpendicular measurements of colony diameter were taken and the mean value was calculated. Results were analyzed by ANOVA and Tukey's statistical tests (Mead et al., 2002). IC_{50} (inhibitory concentration for 50% diameter growth reduction) values for compound **1–3** and extracts were calculated by probit analysis based on the diameter growth of the mycelium bioassays.

4.7. Spectral data of compounds

4.7.1. Preussomerin EG_1 (*1*)

Yellow solid, mp 215.7 °C (decomp); $[\alpha]_D$ −114 (CH_2Cl_2; *c* 1); UV (CH_2Cl_2) λ_{max} nm (log ε) 348 (3.10), 307 (2.72), 264 (3.29), 252 (3.24), 232 (3.66); IR υ_{max} (KBr) 3078, 1679, 1642, 1592, 1472, 1359, 1330, 1297, 1274, 1231 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; EIMS *m*/*z* 348 [M^+ (81)], 303 (4), 191 (4), 175 (31), 174 (100), 173 (4), 147 (8), 146 (11), 118 (6) 75 (7); HRMS *m*/*z* 348.0631 (calcd for $C_{20}H_{12}O_6$, 348.0633).

4.7.2. Preussomerin EG_2 (*2*)

Yellow crystalline needles, mp 224 °C (decomp); $[\alpha]_D$ −143 (MeOH; *c* 1); UV (MeOH) λ_{max} nm (log ε) 359 (3.10), 333 (2.94), 318 (3.04), 283 (2.63), 258 (3.66), 243 (3.49), 221 (3.95); CD (MeOH) Δε (nm) -1.0×10^6 (219) -4.8×10^4 (334); IR υ_{max} (KBr) 3452, 1692, 1648, 1596, 1471, 1329, 1291, 1226 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; EIMS *m*/*z* 366 [M^+ (100)], 348 (7), 337 (18), 323 (6), 295 (6), 282 (12), 277 (6), 266 (6), 238 (13), 192 (23), 176 (20), 175 (10), 174 (11), 163 (9), 147 (6), 91 (8), 55 (8), 18 (26); HRMS *m*/*z* 366.0737 (calcd for $C_{20}H_{14}O_7$ 366.0739).

4.7.3. Preussomerin EG_3 (*3*)

Orange solid, mp 183.6–185.6 °C; $[\alpha]_D$ −178 (MeOH; *c* 1); UV (MeOH) λ_{max} nm (log ε) 362 (3.71), 335 (3.17), 316 (3.31), 283 (3.04), 261 (3.89), 209 (2.53); CD ($CHCl_3$) Δε (nm) -7.3×10^4 (334); IR υ_{max} (KBr) 3375, 2929, 2856, 1739, 1696, 1651, 1598, 1471, 1412, 1361, 1332, 1289, 1262, 1228 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; EIMS *m*/*z* 380 [M^+ (100)], 365 (10), 347 (7), 321 (7), 294 (8), 293 (10), 277 (7), 266 (8), 265 (8), 238 (23), 210 (5), 192 (6), 189 (6), 175 (14), 174 (10), 147 (4), 119 (4), 91 (6), 75 (5), 55 (6); HRMS *m*/*z* 380.0892 (calcd for $C_{21}H_{16}O_7$, 380.0896).

4.7.4. Acetylation of preussomerin EG_1

Preussomerin EG_1 (**1**) (30 mg) was acetylated using Ac_2O (0.5 ml) in pyridine (0.5 ml), with work up as usual. The reaction mixture was purified by TLC (CH_2Cl_2–MeOH; 99:1) to yield monoacetylate product (15 mg) and diacetylated derivative (12 mg). Monoacetylpreussomerin EG_1 (**1a**); pale yellow solid, ^1H NMR ($CDCl_3$, 500 MHz) 2.37 (3H, *s*, $OCOCH_3$-9), 2.56 (1H, *ddd*, *J* = 13.5, 13.5, 5.5 Hz, H-3a), 2.85 (1H, *ddd*, *J* = 19.0, 5.5, 2.0 Hz, H-2a), 2.73 (1H, *ddd*, *J* = 13.5, 5.5, 2.0 Hz, H-3b), 3.24 (1H, *ddd*, *J* = 19.0, 13.5, 5.5 Hz, H-2b), 6.58 (1H, *d*, *J* = 9.5 Hz, H-2′), 7.03 (1H, *d*, *J* = 9.0 Hz, H-7), 7.05 (1H, *dd*, *J* = 8.0, 1.0 Hz, H-9′), 7.08 (1H, *d*, *J* = 9.0 Hz, H-8), 7.18 (1H, *d*, *J* = 9.5 Hz, H-3′), 7.55 (1H, *dd*, *J* = 8.0, 7.5 Hz, H-8′), 7.61 (1H, *dd*, *J* = 7.5, 1.0 Hz, H-7′); ^{13}C NMR ($CDCl_3$, 125 MHz) 21.0 ($OCOCH_3$-9), 32.2 (C-3), 34.1 (C-2), 89.7 (C-4′), 93.8 (C-4), 113.5 (C-10), 117.6 (C-5), 120.2 (C-5′), 120.5 (C-7′), 121.0 (C-9′), 123.0 (C-8), 126.5 (C-7), 130.0 (C-8′), 130.4 (C-10′), 133.5 (C-2′), 141.0 (C-3′), 141.2 (C-6), 147.1 (C-9), 149.5 (C-6′), 169.8 ($OCOCH_3$-9), 184.0 (C-1′), 193.8 (C-1). Diacetylpreussomerin EG_1 (**1b**); pale yellow solid, ^1H NMR ($CDCl_3$, 500 MHz) 2.26 (3H, *s*, $OCOCH_3$-3′), 2.36 (3H, *s*, $OCOCH_3$-9), 2.52 (1H, *ddd*, *J* = 13.2, 13.2, 6.0 Hz, H-3a), 2.84 (1H, *ddd*, *J* = 19.1, 6.0, 1.5 Hz, H-2a), 2.71 (1H, *ddd*, *J* = 13.2, 6.0, 1.5 Hz, H-3b), 3.04 (1 H, *dd*, *J* = 18.3, 3.0 Hz, H-2a′), 3.24 (1H, *ddd*, *J* = 19.1, 13.2, 6.0 Hz, H-2b), 3.41 (1H, *dd*, *J* = 18.3, 3.0 Hz, H-2b′), 5.81 (1H, *t J* = 3.0 Hz, H-3′), 7.03 (1H, *d*, *J* = 9.0 Hz, H-7), 7.10 (1H, *d*, *J* = 9.0 Hz, H-8), 7.10 (1H, *dd*, *J* = 8.0, 1.0 Hz, H-7′), 7.11 (1H, *t*, *J* = 8.0 Hz, H-8′), 7.64 (1H, *dd*, *J* = 8.0, 1.0 Hz, H-9′); ^{13}C NMR ($CDCl_3$, 125 MHz) 20.7 ($OCOCH_3$-3′), 20.9 ($OCOCH_3$-9), 32.2 (C-3), 34.0 (C-2), 40.4 (C-2′), 66.8 (C-3′), 92.8 (C-4′), 93.7 (C-4), 113.7 (C-10), 117.7 (C-5), 119.5 (C-5′), 120.4 (C-9′), 121.9 (C-8′), 122.0 (C-7′), 122.8 (C-8), 126.5 (C-7), 131.2 (C-10′), 144.0 (C-6), 146.7 (C-9), 150.9 (C-6′), 168.6 ($OCOCH_3$-3′), 169.8 ($OCOCH_3$-9), 193.7 (C-1′), 194.0 (C-1).

4.8. X-ray crystallographic analysis of palmarumycin CP_2 (*4*)

Empirical formula $C_{20}H_{14}O_4$, Mr = 318.31, 0.40 × 0.17 × 0.14 mm, colorless prism, from CH_2Cl_2 triclinic, $P\bar{1}$ (No. 2), *a* = 8.5182(6) Å, *b* = 12.7753(8) Å, *c* = 15.1233(10) Å, α = 65.4740(10)°, β = 79.9160(10)°.

$\gamma = 86.8040(10)°$, $V = 1473.87(17)$ Å3, $Z = 4$, $D_{cal} = 1.435$ Mg/m^3, $m\mu = 0.100$ mm^{-1}, $F(000) = 664$, Temp = 298(2) K, Mo Kα $\lambda = 0.71073$ Å, 1.50–25.00° of θ collection, Bruker smart program APEX AXS CCD area detector, the data reduction was carried out with the SAINT program (Bruker, 2001), 12119 reflections were collected, of which 5176 ($R_{int} = 0.0341$) were independent reflections. The structure was solved and refined using full-matrix least-squares on F^2 on the programs SHELXS-2006 (Sheldrick, 1997) and SHELXL-97 (Sheldrick, 1997), respectively. The program XSHELL was used as an interface to the SHELX programs, and to prepare the figures. Two independent molecules were found in the unit cell. The final values of $S = 0.899$, $R1 = 0.0373$, $wR_2 = 0.0787$, were based on 5176 reflections observed with $[I > 2\sigma(I)]$, 439 parameters, the largest diff. peak and hole were 0.143 and -0.183 e Å^{-3}.

5. Supplementary data

Crystallographic data have been deposited at the Cambridge Crystallographic Data Center (CCDC) (CCDC 651731).

Acknowledgements

This work was supported by Grant IN217603 DGAPA-UNAM. We wish to thank Dr. James J. Sims for his valuable comments and corrections to the manuscript; To Ing. Abel Blancas from Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM) for his support in the fermentation of the fungus; To Luis Velasco-Ibarra, Beatriz Quiroz, Javier Pérez Flores, Rocío Patiño, Eréndira García, and Héctor Ríos from Instituto de Química, UNAM for recording MS, CD, IR, UV, NMR spectra; To Carmen Loyola for her valuable help with photos of fungi, and to Claudio Meléndez González for his help in the chemistry work.

References

Anaya, A.L., Torres-Barragán, A., Hernández-Bautista, B.E., Cruz-Ortega, R., Saucedo-García, A., Flores-Carmona, M.C., Gómez-Pompa, A., 2003a. Bioprospection studies at El Eden: From plants to fungi. In: Gómez-Pompa, A., Allen, M.F., Fedick, S.L., Jiménez-Osornio, J.J. (Eds.), The Lowland Maya Area: three Millenia at the Human–Wildland Interface. Food Products Press. The Haworth Press, Inc., New York, pp. 447–460.

Anaya, A.L., Mata, R., Sims, J., González-Coloma, A., Cruz-Ortega, R., Guadaño, A., Hernández-Bautista, B.E., Ríos, G., Gómez-Pompa, A., 2003b. Allelochemical potential of *Callicarpa acuminata* (Verbenaceae). Journal of Chemical Ecology 29, 2725–2740.

Anaya, A.L., Macías-Rubalcava, M.L., Cruz-Ortega, R., García-Santana, C., Sánchez-Monterrubio, P., Hernández-Bautista, B.E., Mata, R., 2005. Allelochemicals from *Stauranthus perforatus,* a rutaceous tree of the Yucatan Peninsula, Mexico. Phytochemistry 66, 487–494.

Arnold, A.E., Maynard, Z., Gilbert, G.S., 2001. Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. Mycological Research 105, 1502–1507.

Bacon, C.W., White, J.F., 2000. Microbial Endophytes. Marcel Dekker, New York.

Barrett, A.G.M., Blaney, F., Campbell, A.D., Hamprecht, D., Meyer, T., White, A.J.P., Witty, D., Williams, D.J., 2002. Unified route to the palmarumycin and preussomerin natural products enantioselective synthesis of (−)-preussomerin G. Journal of Organic Chemistry 67, 2735–2750.

Bode, H.B., Walker, M., Zeeck, A., 2000. Secondary metabolites by chemical screening, 42 Cladospirones B to I from *Sphaeropsidales sp* F 24707 by variation of culture conditions. European Journal of Organic Chemistry, 3185–3193.

Bringmann, G., Busemann, S., Krohn, K., Beckmann, K., 1997. Quantumchemical calculation of CD spectra: the absolute configuration of palmarumycins CP_3 and C_2. Tetrahedron 53, 1655–1664.

Bruker, 2001. SMART and SAINT, Bruker AXS Inc., Madison, Wisconnsin, USA.

González, M.C., Anaya, A.L., Glenn, A.E., Saucedo-García, A., Macías-Rubalcava, M.L., Hanlin, R.T., 2007. A new endophytic ascomycete from El Eden Ecological Reserve, Quintana Roo, Mexico. Mycotaxon 101, 251–260.

Gunatilaka, A.A.L., 2006. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. Journal of Natural Products 69, 509–526.

Hanlin, R.T., Ulloa, M., 1988. Atlas of Introductory Mycology, second ed. Hunter Textbooks, Winston-Salem.

Hu, H., Guo, H., Li, E., Liu, X., Zhou, Y., Che, Y., 2006. Decaspirones F-I, bioactive secondary metabolites from the saprophytic fungus *Helicoma wiridis*. Journal of Natural Products 69, 1672–1675.

Jiao, P., Swenson, D.C., Gloer, J.B., Campbell, J., Shearer, C.A., 2006. Decaspirones A–E, bioactive spirodioxynaphthalenes from the freshwater aquatic fungus *Decaisnella thyridioides*. Journal of Natural Products 69, 1667–1671.

Krohn, K., Michel, A., Florke, U., Aust, H.-J., Drager, S., Schulz, B., 1994a. Palmarumycins CP_1–CP_4 from *Coniothyrium palmarum*: isolation, structure elucidation and biological activity. Liebigs Annalen der Chemie, 1093–1097.

Krohn, K., Michel, A., Florke, U., Aust, H.-J., Draeger, S., Schulz, B., 1994b. Palmarumycins C_1–C_{16} from *Coniothyrium sp.*: isolation, structure elucidation, and biological activity. Liebigs Annalen der Chemie, 1099–1108.

Krohn, K., Beckmann, K., Aust, H.-J., Draeger, S., Schulz, B., Busemann, S., Bringmann, G., 1997a. Biologically active metabolites from fungi, 10 generation of the palmarumycin spiroacetal framework by oxidative cyclization of an open chain metabolite from *Coniothyrium palmarum*. Liebigs Annalen Recueil, 2531–2534.

Krohn, K., Beckmann, K., Flörke, U., Aust, H.-J., Draeger, S., Schulz, B., Busemann, S., Bringmann, G., 1997b. Biologically active metabolites from fungi, 9 New palmarumycins CP_{4a} and CP_5 from *Coniothyrium palmarum*: Structure elucidation, crystal structure analysis and determination of the absolute configuration by CD calculations. Tetrahedron 53, 3101–3110.

Krohn, K., Flörke, U., John, M., Root, N., Steingrover, K., Aust, H.-J., Draeger, S., Schulz, B., Antus, S., Simonyi, M., Zsila, F., 2001. Biologically active metabolites from fungi Part 16. New preussomerins J, K and L from an endophytic fungus: structure elucidation, crystal structure analysis and determination of absolute configuration by CD calculations. Tetrahedron 57, 4343–4348.

Krohn, K., 2003. Natural products derived from naphthalenoid precursors by oxidative dimerization. In: Herz, W., Falk, H., Kirby, G.W., Moore, R.E., Tamm, Ch. (Eds.), Progress in the Chemistry of Organic Natural Products, vol. 85. Springer, Wien, New York, pp. 1–49.

Lazo, J.S., Tamura, K., Vogt, A., Jung, J-K., Rodriguez, S., Balachandran, R., Day, B.W., Wipf, P., 2001. Antimitotic actions of a novel

analog of the fungal metabolite palmarumycin CP_1. The Journal of Pharmacology and Experimental Therapeutics 296, 364–371.

Macías-Rubalcava, M.L., Hernández-Bautista, B.E., Jiménez-Estrada, M., Anaya, A.L., 2007. Pentacyclic triterpenes with selective bioactivity from the leaves of *Sebastiania adenophora*, Euphorbiaceae. Journal of Chemical Ecology 33, 147–156.

McDonald, L.A., Abbanat, D.R., Barbieri, L.R., Beruan, V.S., Discafani, C.M., Greenstein, M., Janota, K., Korshalla, J.D., Lassota, P., Tischler, M., Carter, G.T., 1999. Spiroxins, DNA cleaving antitumor antibiotics from a marine-derived fungus. Tetrahedron Letters 40, 2489–2492.

Mead, R., Curnow, R.N., Hasted, A.M., 2002. Statistical Methods. In: Agriculture and Experimental Biology, third ed. Chapman and Hall, CRC, Boca Raton, FL.

Prajoubklang, A., Sirithunyalug, B., Charoenchai, P., Suvannakad, R., Sriubolmas, N., Piyamongkol, S., Kongsaeree, P., Kittakoop, P., 2005. Bioactive deoxypreussomerins and dimeric naphthoquinones from *Diospyros ehretioides* fruits: deoxypreussomerins may not be plant metabolites but may be from fungal epiphytes or endophytes. Chemistry and Biodiversity 2, 1358–1367.

Quesada, E., Stockley, M., Ragot, J.P., Prime, M.E., Whitwood, A.C., Taylor, R.J.K., 2004. A versatile, non-biomimetic route to the preussomerins: syntheses of (+/−)-preussomerins F, K and L. Organic and Biomolecular Chemistry 2, 2483–2495.

Ragot, J.P., Steeneck, C., Alcaraz, M-L., Taylor, R., 1999. The synthesis of 1,8-dihydroxynaphthalene-derived natural products: palmarumycin CP1, palmarumycin CP2, palmarumycin C11, CJ- 12,371, deoxypreussomerin A and novel analogs. Journal of Chemical Society Perkin Transactions 1, 1073–1082.

Rodríguez, K.F., 1994. The foliar fungal endophytes of the Amazonian palm *Euterpe oleracea*. Mycologia 86, 376–385.

Rodríguez, K.F., 1996. Fungal endophytes of palms. In: Redlin, S.C., Carris, L.M. (Eds.), Endophytic Fungi in Grasses and Woody Plants, Systematics , Ecology and Evolution. ASP Press, St. Paul, MN, pp. 121–132.

Sakemi, S., Inagaki, T., Kaneda, K., Hirai, H., Iwata, E., Sakakibara, T., Yamauchi, Y., Norcia, M., Wondrack, L.M., Sutcliffe, J.A., Kojima, N., 1995. CJ-12,371 and CJ-12,372, two novel DNA gyrase inhibitors Fermentation, isolation, structural elucidation and biological activities. The Journal of Antibiotics 48, 134–142.

Schulz, B., Römmert, A.-K., Dammann, U., Aust, H.-J., Strack, D., 1999. The endophyte-host interaction: a balanced antagonism? Mycological Research 103, 1275–1283.

Schulz, B., Boyle, C., Draeger, S., Römmert, A.K., Krohn, K., 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycological Research 106, 996–1004.

Schulz, B., Boyle, C., 2005. The endophytic continuum. Mycological Research 109, 661–686.

Seephonkai, P., Isaka, M., Kittakoop, P., Palittapongarnpim, P., Kamchonwongpaisan, S., Tanticharoen, M., Thebtaranonth, Y., 2002. Evaluation of antimycobacterial, antiplasmodial and cytotoxic activities of preussomerins isolated from the lichenicolous fungus *Microsphaeropsis* sp. BCC 3050. Planta Medica 68, 45–48.

Sheldrick, G.M., 1997. SHELXL97and SHELXS97, University of Göttingen, Germany; SHELXTL/PC. Version 5.101. Bruker AXS Inc., Madison, Wisconsin, USA.

Singh, S.B., Zink, D.L., Liesch, J.M., Ball, R.G., Goetz, M.A., Bolessa, E.A., Giacobbe, R.A., Silverman, K.C., Bills, G.F., Pelaez, F., Cascales, Gibbs, J.B., Lingham, R.B., 1994. Preussomerins and deoxypreussomerins: Novel inhibitors of ras farnesyl-protein transferase. Journal of Organic Chemistry 59, 6296–6302.

Soman, A.G., Gloer, J.B., Koster, B., Malloch, D., 1999. Sporovexins A–C and a new preussomerin analog: antibacterial and antifungal metabolites from the coprophilous fungus *Sporormiella vexans*. Journal of Natural Products 62, 659–661.

Stone, J.K., Polishook, J.D., White, J.F., 2004. Endophytic fungi. In: Mueller, G.M., Bills, G.F., Foster, M.S. (Eds.), Biodiversity of fungi: Inventory and monitoring methods. Elsevier Academic Press, China, pp. 241–270.

Strobel, G.A., 1996. Endophytic fungi: New sources for old and new pharmaceuticals. Pharmaceutical News 3, 7–9.

Strobel, G., 2006. Harnessing endophytes for industrial microbiology. Current Opinion in Microbiology 9, 240–244.

Strobel, G.A., Daisy, B., 2003. Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews 67, 491–502.

Strobel, G.A., Long, D.M., 1998. Endophytic microbes embody pharmaceutical potential. ASM News 64, 263–268.

Strobel, G.A., Daisy, B., Castillo, U., Harper, J., 2004. Natural products from endophytic microorganisms. Journal of Natural Products 67, 257–268.

Tan, R.X., Zou, W.X., 2001. Endophytes: a rich source of functional metabolites. Natural Product Reports 18, 448–459.

Vilella, D., Sánchez, M., Platas, G., Salazar, O., Genilloud, O., Royo, I., Cascales, C., Martín, I., Díez, T., Silverman, K.C., Lingham, R.B., Singh, S.B., Jayasuriya, H., Peláez, F., 2000. Inhibitors of farnesylation of Ras from a microbial natural products screening program. Journal of Industrial Microbiology and Biotechnology 25, 315–327.

Weber, H.A., Baenziger, N.C., Gloer, J.B., 1990. Structure of preussomerin A: an unusual new antifungal metabolite from the coprophilous fungus Preussia isomera. Journal of American Chemical Society 112, 6718–6719.

Weber, H.A., Gloer, J.B., 1991. The preussomerins: novel antifungal metabolites from the coprophilous fungus Preussia isomera Cain. Journal of Organic Chemistry 56, 4355–4360.

Wipf, P., Jung, J-K., Rodriguez, S., Lazo, J.S., 2001a. Synthesis and biological evaluation of deoxypreussomerin A and palmarumycin CP1 and related naphthoquinone spiroketals. Tetrahedron 57, 283–296.

Wipf, P., Hopkins, T.D., Jung, J-K., Rodriguez, S., Birmingham, A., Southwick, E.C., Lazo, J.S., Powis, G., 2001b. New inhibitors of the thioredoxin–thioredoxin reductase system based on a naphthoquinone spiroketal natural product lead. Bioorganic and Medicinal Chemistry Letters 11, 2637–2641.

Wipf, P., Lynch, S.M., Birmingham, A., Tamayo, G., Jiménez, A., Campos, N., Powis, G., 2004. Natural product based inhibitors of the thioredoxin–thioredoxin reductase system. Organic and Biomolecular Chemistry 2, 1651–1658.

Wipf, P., Lynch, S.M., Powis, G., Birmingham, A., Englund, E.E., 2005. Synthesis and biological activity of prodrug inhibitors of the thioredoxin–thioredoxin reductase system. Organic and Biomolecular Chemistry 3, 3880–3882.

Wiyakrutta, S., Sriubolmas, N., Panphut, W., Thongon, N., Danwisetkanjana, K., Ruangrungsi, N., Meevootisom, V., 2004. Endophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants. World Journal of Microbiology and Biotechnology 20, 265–272.