Pergamon

Tetrahedron 56 (2000) 5337–5344

TETRAHEDRON

Conformational Behavior and Absolute Stereostructure of Two Phytotoxic Nonenolides from the Fungus *Phoma herbarum*☆

José Fausto Rivero-Cruz,[a] Genoveva García-Aguirre,[b,*] Carlos M. Cerda-García-Rojas[c] and Rachel Mata[a,*]

[a]*Facultad de Química, Universidad Nacional Autónoma de México, México, DF 04510, Mexico*
[b]*Instituto de Biología, Universidad Nacional Autónoma de México, México, DF 04510, Mexico*
[c]*Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado 14-740, México, DF 07000, Mexico*

Received 29 March 2000; accepted 28 May 2000

Abstract—Bioactivity-directed fractionation of extracts from the fungus *Phoma herbarum* led to the isolation of two new phytotoxic nonenolides: (7*S*,8*S*,9*R*)-7,8-dihydroxy-9-propyl-5-nonen-9-olide (**1**) and (2*R*,7*S*,8*S*,9*R*)-2,7,8-trihydroxy-9-propyl-5-nonen-9-olide (**2**), which were named herbarumins I and II, respectively. The stereostructures were elucidated by spectroscopic methods and a combination of molecular modeling, NOESY and $^{1}H-^{1}H$ coupling constant data, which revealed that in $CDCl_3$ solution, **1** exists in one preferred conformation, while **2** exhibits a conformational equilibrium. Compounds **1** and **2** caused significant inhibition of radicle growth of seedlings of *Amaranthus hypochondriacus*.

Introduction

The genus *Phoma* comprises about 2000 species, many of which are phytopathogenic causing severe diseases on several economically important crops characterized by necrotic lesions on leaves, stems and fruits.[1] Some of the phytopathogenic species have yielded important phytotoxins such as phomalirazine,[2] the betaenones[3] the altiltoxins,[4] epoxydon,[5] and the putaminoxins[6,7] among others.

As a part of our search for potential herbicidal agents from Mexican plants and microorganisms, we describe in this investigation the isolation and structural elucidation of the major phytotoxic principles from the culture broth and mycelium of an isolate of *Phoma herbarum* Westend (Sphaeropsidaceae) [syn. *Phoma pigmentivora* Massee].[8] *P. herbarum* has a worldwide distribution and is known from a variety of substrates including herbaceous and woody plants, soil and water. This fungus inhibits the growth of the alga *Chlorella pyrenoidosa* in vitro and is pathogenic to *Avena fatua*[1] and dandelion[8] (*Taraxacum officinale*) seedlings after artificial infection. Furthermore, *P. herbarum* was recovered and characterized from small necrotic lesions on dandelion foliage.[8] Chemical work on this species led to the isolation of the anthraquinones helminthosporin and 1-hydroxyhelminthosporin (cynodontin) and the isomeric chromanones LL-D253α, LL-D253β and LL-D253γ.[1,9–11]

Results and Discussion

The fungus *P. herbarum* was grown in liquid-substrate fermentation on modified M-D-I medium.[12] The culture broth and mycelium were extracted with EtOAc. The extract showed phytotoxic activity (Table 1) when evaluated on seedlings of *Amaranthus hypochondriacus* L. using a Petri dish bioassay.[13] Bioactivity-guided fractionation of this extract led to the isolation of the new nonenolides **1** and **2**, which were given the trivial names of herbarumins I and II, respectively.

Table 1. Effect of the extract and isolated compounds from *Phoma herbarum* on radicle elongation of *A. hypochondriacus*

Compound	IC_{50} (M)
Extract[a]	35.80
1	5.43×10^{-5}
2	1.25×10^{-4}
4	1.47×10^{-3}
5	2.75×10^{-3}
2,4-D[b]	1.92×10^{-4}

[a] Expressed in μg/mL.
[b] 2,4-dichlorophenoxyacetic acid as the positive control.

☆ Taken in part from the Ph.D. thesis of José F. Rivero-Cruz.

Keywords: nonenolides; lactones; fungal metabolites; fungi; *Phoma herbarum*; configuration; conformation; phytotoxic activity.
* Corresponding authors. Tel.: +52-5622-5289; fax: +52-5622-5329; e-mail: rachel@servidor.unam.mx or ccerda@mail.cinvestav.mx

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00469-5

Table 2. 1D and selected 2D NMR chemical shift assignment of herbarumin I (**1**) (measured at 125 MHz for ^{13}C NMR and 500 MHz for ^{1}H in $CDCl_3$)

Position	^{13}C δ[a]	^{1}H δ[b]	HMBC
1	176.4	–	2a, 2b, 3a, 9
2a	34.4	2.32 ddd, 14.0, 6.0, 2.0	3a, 3b, 4a, 4b
2b		2.01 ddd, 14.0, 12.5, 2.0	
3a	24.6	1.89 m	2a, 2b, 4a, 4b
3b		1.73 m	
4a	33.3	2.40 brdt, 12.0, 3.0	3a, 3b, 5
4b		1.97 m	
5	124.8	5.52 dddd, 15.5, 10.0, 4.0, 2.5	2a, 4b, 7
6	130.7	5.62 brd, 15.5, 2.5	4a, 4b, 5, 7
7	73.3	4.43 quint, 2.0	6
8	73.6	3.52 dd, 9.8, 2.5	6, 7, 9
9	70.2	4.96 td, 9.8, 2.5	7, 8, 10a, 10b
10a	33.7	1.87 m	8, 11
10b		1.55 m	
11a	17.9	1.38 m	9, 10a, 10b, 12
11b		1.30 m	
12	13.8	0.89 t, 7.5	10a, 10b, 11a, 11b

[a] Assignments confirmed by HMQC and HMBC.
[b] Coupling constants are in Hz.

Herbarumin I (**1**) was shown to have the molecular formula of $C_{12}H_{20}O_4$ (three unsaturations) from HREIMS, based on a molecular ion peak at *m/z* 228.1364. IR spectroscopy indicated the presence of hydroxyl (3390 cm^{-1}) and olefin functionalities (1670 cm^{-1}), in addition to the lactone group (1721 cm^{-1}). The NMR data coupled with the IR and MS indicated that herbarumin I (**1**) has a structure similar to those of pinolidoxin and related nonenolides.[6,7,14] In particular the NMR spectra (Table 2) showed the existence of a ten-membered macrolide core, a vicinal diol, a *trans* disubstituted double bond, and an *n*-propyl unit. COSY and HMBC experiments indicated the position of the hydroxyl, *n*-propyl and olefin functionalities on the macrolactone core. Thus, the HMBC correlations C-5/H-7 and H-4b, C-7/H-6, C-8/H-6 and H-7, and C-9/H-7 and H-8 were consistent with the placement of the double bond and the diol grouping at C-5/C-6 and C7/C-8, respectively. In addition, the correlations C-9/H-10a and H-10b, and C-1/H-9 revealed that the *n*-propyl moiety and the lactone closure were located at C-9.

The stereochemistry and the solution conformation of the ten-membered ring lactone of herbarumin I (**1**) was determined by molecular mechanics modeling,[15] NOESY data and the comparison of the observed vs. the calculated vicinal proton coupling constants. Thus, a systematic conformational search for this substance using the MMX force field as implemented in the PCMODEL program revealed the presence of the minimum energy conformation depicted in Fig. 1 (E_{MMX}=9.51 kcal/mol), which was in agreement with the NOESY correlations also indicated in Fig. 1. The dihedral angles measured in this molecular model were converted to calculated coupling constants by means of a generalized Karplus-type relationship[16,17] and compared with the experimental coupling constants (Table 4). The close correlation between the calculated and experimental *J* values denoted that the ten-membered ring of herbarumin I (**1**) exists in a single conformation, which is related to the chair–chair–chair conformation found in cyclodecane.[18] In this conformation, the value of the coupling constant (J=2.5 Hz) between H-7 and H-8 indicated their *cis*-equatorial–axial relationship. On the other hand, the value of the coupling constant (J=9.5 Hz) between H-8 and H-9 revealed their *trans*-diaxial relationship, as in the case of pinolidoxin.[6] Concerning the *n*-propyl side-chain, the observed vs. the calculated coupling constant values (Table 4) supported the proposition that in solution, the C(8)–C(9)–C(10)–C(11) torsion angle is ca. 180°, as in the calculated minimum energy conformation. The absolute configuration at C-7 and C-8 was determined using the CD exciton coupling method of Harada and Nakanishi.[19,20] Treatment of **1** with *p*-bromobenzoyl chloride gave the di-*p*-bromobenzoate derivative **3**, whose minimum energy conformation (E_{MMX}=49.67 kcal/mol) was similar to that of **1**. The observed coupling constants $J_{7,8}$=2.0 and $J_{8,9}$=9.3 Hz (Table 4) were also in agreement with the calculated conformation. The CD spectrum of **3** indicated a bisignate [-6.42×10^4 (237), 9.26×10^5 (255)] Cotton effect, corresponding to a positive chirality. Thus, the stereogenic centers at C-7 and C-8 were each determined to have the *S*-configuration. The absolute configuration at C-9 was therefore assigned as *R*. On the basis of the above evidence, herbarumin I (**1**) was identified as (7*S*,8*S*,9*R*)-7,8-dihydroxy-9-propyl-5-nonen-9-olide.

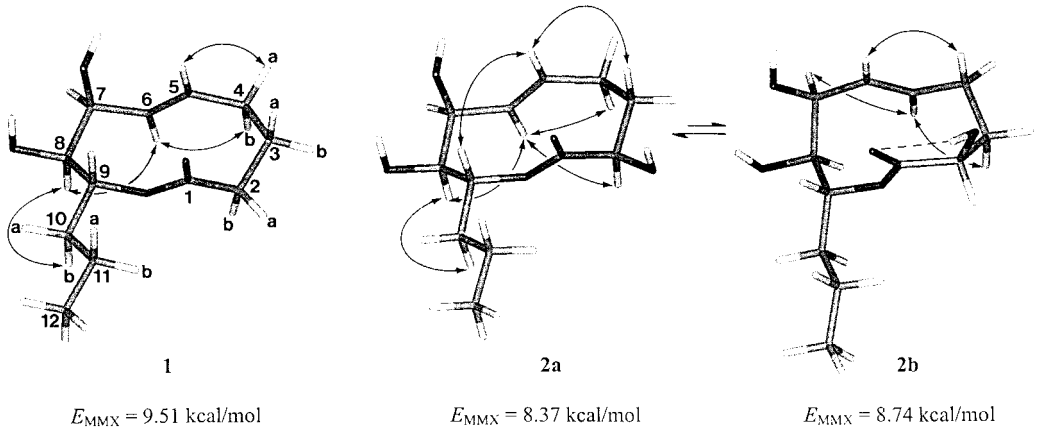
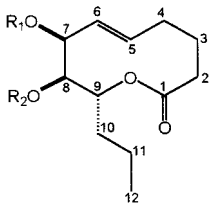
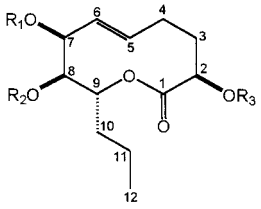


Figure 1. Minimum energy structures of herbarumin I (**1**) and herbarumin II (**2a** and **2b**), showing relevant NOESY correlations.



$R_1 = R_2 = H$ (**1**)
$R_1 = R_2 = BrBz$ (**3**)

Herbarumin II (**2**) had the composition $C_{12}H_{20}O_5$ (three unsaturations) as determined by HREIMS, differing from **1** by 16 mass units. This observation as well as the NMR data (Table 3) suggested that **2** was the C-2 hydroxyl derivative of **1**. Treatment of **2** with pyridine/$(CH_3CO)_2O$ yielded the triacetyl derivative **4** confirming the presence of three hydroxyl groups in the molecule. In addition, the presence of a vicinal diol in **2** was corroborated by obtaining the acetonide **5** upon treatment of **2** with anhydrous Me_2CO and $CuSO_4$ (reflux). The NMR spectra of **2** were almost identical with those of **1** except for the presence of signals for an additional secondary carbinol group (δ_H/δ_C 3.99, dd, J=8.2, 3.0 Hz/72.6) in place of the methylene signals attributed to C-2. As in the case of **1** the disposition of the substituents along the macrolactone core was established by the analysis of the COSY and HMBC spectra.



$R_1 = R_2 = R_3 = H$ (**2**)
$R_1 = R_2 = R_3 = Ac$ (**4**)
$R_1 + R_2 = (CH_3)_2C$, $R_3 = H$ (**5**)
$R_1 = R_2 = R_3 = BrBz$ (**6**)

During the NMR spectral analysis of herbarumin II (**2**) in $CDCl_3$, the presence of a small group of signals indicated the existence of a second component in (~86:14). Further purification of **2** always led to the same signal pattern and proportion. However, when the spectrum of **2** was recorded using MeOH-d_4, only one component was detected. On repeating the determination in $CDCl_3$, the original mixture was obtained. These observations were consistent with a conformational equilibrium between two species. It appears that in $CDCl_3$ solution their interconversion at room temperature was slower than the NMR time-scale, allowing its independent observation. Molecular mechanics calculations[15] were used again to assist the rationalization of the experimental data (NOESY and coupling constants) in an effort to describe in detail the conformational properties of compound **2**. The results indicated the presence of two minimum energy conformations, which are represented in Fig. 1. The ten-membered ring in **2a** (E_{MMX}=8.37 kcal/mol) exists in a chair–chair–chair conformation very similar to that found in **1**, while this ring in **2b** (E_{MMX}=8.74 kcal/mol) resembled the twist chair–boat–chair conformation of cyclodecane.[18] The NOESY spectrum of **2** exhibited the interactions shown in Fig. 1, which were in agreement with the presence of both conformations. In particular, the interaction between H-2 and H-6 in **2a** was also consistent with a pseudoequatorial (β) disposition of the hydroxyl group at C-2. It is clear that in $CDCl_3$ solution, the formation of the O(2)H–O(1) hydrogen bond accounts for the stabilization of conformer **2b**, while in MeOH-d_4, this intramolecular hydrogen bonding is released by interaction and exchange of the labile hydrogen O(2)H with the solvent atoms, as revealed by the presence of a single conformer (**2a**) in the ^{1}H NMR spectrum. In MeOH-d_4, the signal for the proton geminal to the C-2 oxygen of **2a** was observed as a sharp dd with $J_{2,3a}$=10.2 Hz and $J_{2,3b}$=3.2 Hz. The ^{1}H NMR spectrum of triacetate **4** showed the same coupling pattern for H-2 (Table 3), suggesting that the ten-membered ring of **4** was also in a single conformation similar to **2a**. A molecular mechanics conformational search for triacetate **4** in combination with the calculated vs. observed coupling constants (Table 4) supported this assumption. The minimum energy structure of **4** had E_{MMX}=5.97 kcal/mol and closely resembled that of **2a**. Interestingly, the ten-membered ring of acetonide **5** was found in a conformation similar to the minor component **2b**, as revealed by the minimum energy conformer (E_{MMX}=24.30 kcal/mol) and by the calculated vs. observed coupling constants listed in Table 4. Additional evidences to support the conformational equilibrium in $CDCl_3$ between **2a** and **2b** were obtained through a series of low temperature DNMR experiments. As can be seen in Fig. 2, when temperature decreases, the amount of conformer **2b** substantially increases. This is consistent with the proposed stabilization of **2b** by the O(2)H–O(1) intramolecular hydrogen bonding. Estimation of ΔG values using the $\Delta G = -RT \ln K$ equation and relevant integration values from the ^{1}H NMR spectra of Fig. 2 afforded $\Delta G^{25°C}$=1.09 kcal/mol and $\Delta G^{-40°C}$=0.40 kcal/mol for the **2a** ⇌ **2b** equilibrium. Interconversion of **2a** in **2b** might proceed via two non-synchronized conformational movements, involving bond rotations of the C(1)–C(2)–C(3)–C(4)–C(5) and C(4)–C(5)=C(6)–C(7) moieties, which would lead to two intermediate conformations. The presence of these conformations (E_{MMX}=8.90 kcal/mol and E_{MMX}=9.90 kcal/mol, respectively) can be envisaged by the reduction of the *trans*-diaxial coupling constants $J_{2b,3a}$ and $J_{4b,5}$ in **2a** (Table 4), on going from MeOH-d_4, where the interconversion process does not take place, to $CDCl_3$.

The absolute configuration of **2** at the stereogenic centers at C-2, C-7, C-8 and C-9 was determined following the same procedure as for compound **1**. The tri-*p*-bromobenzoate **6**, whose ten-membered ring conformation (E_{MMX}=67.34 kcal/mol) resembled those of **1**, **2a**, **3** and **4** (Table 4), showed a typical split CD and this established the chirality of the 7-OBrBz/8-OBrBz as being positive.[20] Therefore, herbarumin II (**2**) was characterized as (2*R*,7*S*,8*S*,9*R*)-2,7,8-trihydroxy-9-propyl-5-nonen-9-olide (**2**). Comparison of the H-2 coupling constants of **4** (10.0 and 3.5 Hz) with those of pinolidoxin (5.6 and 1.7 Hz)[6] suggested that the stereochemistry at C-2 in both compounds might be opposite, although a conformational study of pinolidoxin would be desirable to confirm this observation.

Natural products **1** and **2** as well as derivatives **4** and **5** were

Table 3. NMR data for herbarumin II (**2**) and triacetylherbarumin II (**4**) (measured at 125 MHz for ^{13}C and 500 MHz for ^{1}H)

	2($CDCl_3$)				**2**(CD_3OD)	**4**($CDCl_3$)	
	2a			**2b**			
Position	^{13}C δ^{a}	HMBC	^{1}H $\delta^{a,b}$	^{1}H $\delta^{a,b}$	^{1}H δ^{b}	^{13}C $\delta^{a,b,d}$	^{1}H $\delta^{a,b,c}$
1	177.0	2, 3a, 9	–	–	–	171.2	–
2	72.6	3a, 3b, 4a, 4b	3.99 dd, 8.2, 3.0	4.27 t, 3.5	3.85 dd, 10.0, 3.2	73.5	4.75 dd, 10.0, 3.5
3a	33.8	2, 4a, 4b	2.05 m	2.03 m	1.92 m	30.6	2.00 m
3b			2.10 m	1.86 m	1.80 m		2.00 m
4a	25.2	3a, 3b, 5	2.34 m	2.27 m	2.32 m	27.9	2.37 m
4b			2.15 m	2.10 m	2.11 m		2.18 dtd, 15.0, 10.0, 5.0
5	122.4	4b, 7	5.59 dddd, 16.3, 8.0, 5.5, 2.5	5.51 dddd, 16.3, 10.5, 4.0,1.0	5.50 dddd, 16.3, 10.0, 5.5, 2.5	123.9	5.44 dddd, 15.5, 10.0, 4.5, 2.0
6	131.9	4a, 4b, 5,7	5.63 dd, 16.3, 2.5	5.39 ddd, 16.5, 6.5, 1.0	5.60 dd, 16.3, 2.5	127.7	5.60 dd, 15.0, 2.5
7	68.3	6	4.48 br s	4.31 dd, 6.5, 3.0	4.35 quint, 2.5	70.7	5.68 quint, 2.5
8	71.8	6, 7	3.56 dd, 9.8, 2.5	3.71 dd, 4.5, 3.0	3.53 dd, 9.8, 2.5	71.8	4.95 dd, 10.0, 2.5
9	70.7	7, 8, 10a, 10b	5.06 td, 9.8, 2.5	5.00 dt, 9.0, 4.5	5.37 td, 9.8, 2.5	70.0	5.37 ddd, 10.0, 8.5, 3.0
10a	33.8	8,11	1.77 m	–	1.86 m	33.4	1.56 m
10b			1.57 m	–	1.55 m		1.46 m
11a	17.8	9, 10a, 10b, 12	1.47 m	–	1.37 m	17.2	1.31 m
11b			1.31 m	–	1.37 m		1.31 m
12	13.8	10a, 10b, 11a, 11b	0.91 t, 7.5	0.93 t, 7.5	0.92 t, 7.5	13.9	0.89 t, 7.5

[a] Assignments confirmed by HMQC and HMBC.
[b] Coupling constants are in Hz.
[c] 2-CH_3CO: 2.17; 7-CH_3CO: 2.09; 8-CH_3CO: 2.04 ppm.
[d] 2-CH_3CO: 20.6, 169.9; 7-CH_3CO: 20.7, 169.9; 8-CH_3CO: 20.8, 169.5 ppm.

Table 4. Selected dihedral angles (ϕ_{MMX} in degrees), calculated coupling constants (J_{calc} in Hz) and observed coupling constants (J_{obs} in Hz) for herbarumin I (**1**), herbarumin II (**2a** and **2b**), 7,8-di-*p*-bromobenzoyl-herbarumin I (**3**), 2,7,8-triacetylherbarumin II (**4**), 7,8-*O,O′*-isopropylideneherbarumin II (**5**) and 2,7,8-tri-*p*-bromobenzoylherbarumin II (**6**)

H(x)–C–C–H(y)	**1**			**2a**				**2b**			**3**			**4**			**5**			**6**		
x,y	ϕ_{MMX}	J_{calc}	J_{obs}[a]	ϕ_{MMX}	J_{calc}	J_{obs}[b]	J_{obs}[a]	ϕ_{MMX}	J_{calc}	J_{obs}[a]	ϕ_{MMX}	J_{calc}	J_{obs}[c]	ϕ_{MMX}	J_{calc}	J_{obs}[a]	ϕ_{MMX}	J_{calc}	J_{obs}[c]	ϕ_{MMX}	J_{calc}	J_{obs}[c]
2a,3a	−68	1.7	2.0	–	–	–	–	–	–	–	−68	1.7	–[d]	–	–	–	–	–	–	–	–	–
2a,3b	+48	5.3	6.0	–	–	–	–	–	–	–	+48	5.3	–[d]	–	–	–	–	–	–	–	–	–
2b,3a	+175	13.6	12.5	+177	11.6	10.2	8.2	−68	2.8	3.5	+175	13.6	–[d]	+176	11.6	10.0	−71	2.4	3.5	+176	11.6	9.6
2b,3b	−69	1.7	2.0	−68	3.1	3.2	3.0	+46	3.6	3.5	−69	1.7	–[d]	−68	2.9	3.5	+43	4.2	3.5	−68	2.9	3.9
4a,5	−57	3.8	4.0	−62	3.5	5.5	5.5	+169	11.3	10.5	−57	3.8	4.0	−60	3.4	4.5	+172	11.4	9.2	−61	3.5	4.0
4b,5	−175	11.5	10.0	−179	11.6	10.0	8.0	+51	4.2	4.0	−173	11.5	10.0	−178	11.6	10.0	+54	4.0	5.4	−179	11.6	10.0
6,7	−53	4.0	2.5	−53	4.0	2.5	2.5	+114	4.1	6.5	−52	4.1	2.0	−54	4.0	2.5	+124	5.4	7.5	−49	4.3	2.0
7,8	+57	2.6	2.5	+57	2.7	2.5	2.5	+51	3.2	3.0	+55	2.9	2.0	+58	2.6	2.5	+38	5.0	6.0	+58	2.6	2.0
8,9	+179	9.3	9.8	−177	9.3	9.8	9.8	+178	9.3	9.0	−177	9.4	9.3	−179	9.4	10.0	−179	9.4	10.3	−179	9.4	10.0
9,10a	+62	2.0	2.5	+62	2.0	2.5	2.5	+60	2.2	4.5[e]	+63	1.8	2.7	+68	1.4	3.0[e]	+62	2.0	2.5[e]	+70	1.3	5.4[e]
9,10b	+179	11.6	9.8	+179	11.6	9.8	9.8	+177	11.6	9.0[e]	+180	11.5	12.0	−175	11.2	8.5[e]	+180	11.5	9.3[e]	−173	11.1	6.3[e]

[a] Measured at 500 MHz in $CDCl_3$.
[b] Measured at 500 MHz in CD_3OD.
[c] Measured at 300 MHz in $CDCl_3$.
[d] Signal overlapping precluded the measurement.
[e] Averaged by contribution of additional rotameric species in the side chain.

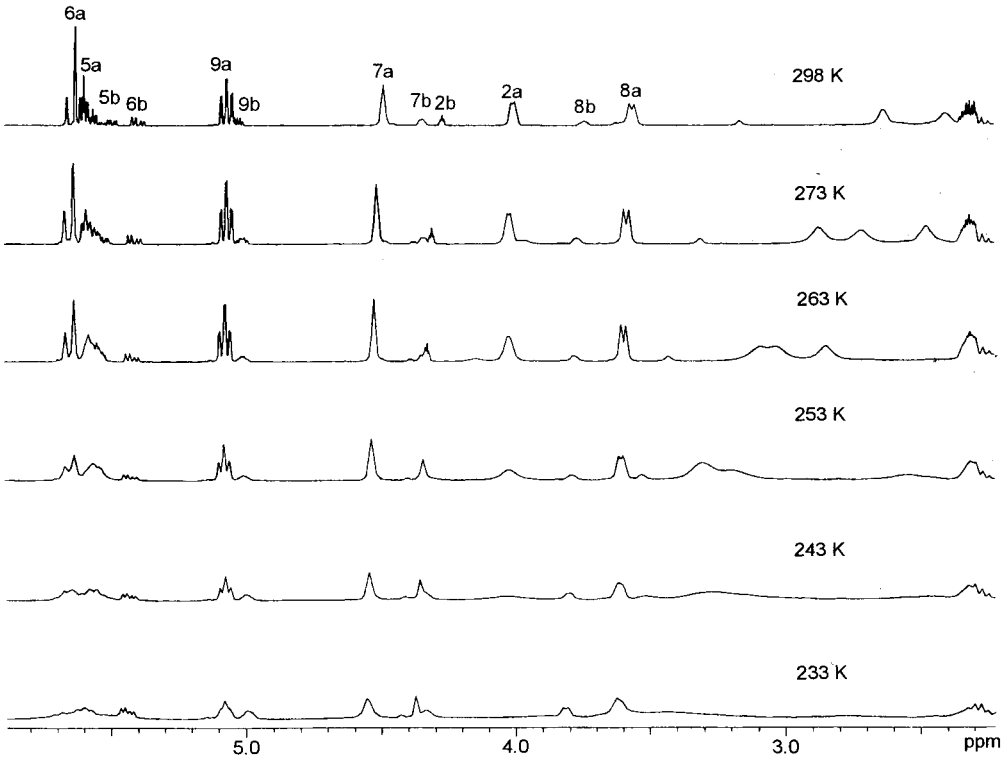


Figure 2. The δ 5.9–2.2 region of the ^{1}H NMR ($CDCl_3$) spectrum of **2** (**2a**+**2b**) recorded at low temperature.

evaluated for their ability to inhibit seed germination and seedling growth of *A. hypochondriacus*. Table 1 summarizes the phytotoxic effect of the tested compounds on seedling growth. The results are expressed as IC_{50} (50% inhibitory concentration). Compound **1** was more potent than the positive control (2,4-D), while compound **2** exhibited a similar potency with respect to 2,4-D. Blocking of the diol functionality in compound **2** significantly decreased the pre-emergent phytotoxic activity. Previously, Evidente et al. demonstrated that the post-emergent phytotoxic effect of pinolidoxin, the toxic agent of the fungus *Ascochyta pinodes*, required not only the integrity of the nonenolide ring and the propyl side chain but also the presence of hydroxyl groups at C-8 and C-9.[21] It is important to point out that the level of activity displayed by **1** clearly revealed its potential as herbicide agent. Finally, this work is intended to be a contribution to the understanding of conformations of hydroxylated ten-membered heterocyclic rings in protic vs. aprotic solvents.

Experimental

General experimental procedures

Melting point determinations were performed on a Fisher–Johns apparatus and are uncorrected. IR spectra were obtained using KBr disks on a Perkin–Elmer 599B spectrophotometer. UV spectra were obtained on a Shimadzu 160 LTV spectrometer in MeOH and EtOH solutions. Optical rotations were taken on a JASCO DIP-360 digital polarimeter. CD spectra were performed on a JASCO 720 spectropolarimeter at 25°C in MeOH and EtOH solutions. NMR spectra including COSY, NOESY, HMBC and HMQC experiments were recorded on a Varian Unity Plus 500 spectrometer or on a Bruker DMX500 in $CDCl_3$ at 500 MHz (^{1}H) or 125 MHz (^{13}C). 300 MHz ^{1}H NMR was registered on a Varian VXR300S instrument. FABMS were recorded on a JEOL DX300 mass spectrometer in the positive mode using NBA as the matrix. EIMS data were obtained on a JEOL JMS-AX505HA mass spectrometer. HPLC was carried out with a Waters HPLC instrument equipped with a Waters UV photodiode array detector (900) set at 209 nm, using a silica gel column (19 mm i.d.×300 mm). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the MILLENNIUM 32 software program (Waters). Open column chromatography: silica gel 60 (70–230 mesh, Merck). Flash column chromatography: silica gel (40 μm, Baker). TLC: silica gel 60 F_{254} (Merck).

Fungal material

The fungus *Phoma herbarum* was isolated from a special variety of *Zea mays* ("maíz cacahuacintle") grown in Michoacán, Mexico, in 1998. Cultures of the isolate are maintained in the mycological collection of Laboratorio de Micopatología, Instituto de Biología, UNAM (Voucher: TOX-01020). Stock cultures of the fungus were stored on agar slants of potato dextrose agar (PDA), with subculturing at monthly intervals. Forty 1.8 L Fernbach flasks, each containing 1 L of modified M-D-I medium that had been sterilized at 120°C for 15 min and then cooled to room temperature, were individually inoculated with 1 cm^2 agar plugs taken from stock cultures of *P. herbarum* maintained at 4°C on PDA. Flask cultures were incubated at 28°C and

aerated by agitation on an orbital shaker at 120 rpm for 15 days.

Extraction and isolation of 1 and 2

After incubation, the content of each flask were combined and filtered. The culture filtrates (40 L) were extracted exhaustively with EtOAc (3×40 L). The combined organic extracts were filtered over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a brown oil (3.2 g). The mycelium was extracted with EtOAc (3×1.5 L). The mycelial extract was evaporated to yield 0.801 g of a dark brown solid. The combined mycelial and broth extracts (4.014 g) were subjected to open column chromatography on silica gel (300 g) and eluted with a gradient of increasing polarity CH_2Cl_2–*i*-PrOH (0–100%). Altogether 229 (250 mL) fractions were collected and then combined according to their TLC patterns to yield eight primary fractions (FI–FVIII). Bioactivity in the bioautographic bioassay showed one active pool (FIV). Fraction FIV, eluted with CH_2Cl_2–*i*-PrOH (90:10), was subjected to flash column chromatography on silica gel (32 g) and eluted with a gradient of increasing polarity of CH_2Cl_2–*i*-PrOH (90:10 to 50:50). 104 fractions were collected and then combined to yield eight fractions (FIV-1–FIV-8). According to the bioautographic bioassay the activity was concentrated in fraction FIV-3, eluted with CH_2Cl_2–*i*-PrOH (85:15). HPLC purification of the active fraction FIV-3 (186.4 mg) on a silica gel column [8.2 mL min^{-1}, hexane–*i*-PrOH–MeOH, (82:9:9)] yielded **1** (12.8 mg) and **2** (30.1 mg). The R_t values were 10.2 and 20.8 min, respectively. The R_f values on silica gel plates, developed with CH_2Cl_2–*i*-PrOH (88:12), were 0.55 and 0.38, respectively. HPLC purification of fraction FIV-4 using the same conditions afforded additional amounts of compounds **1** (3.2 mg) and **2** (67.0 mg).

Herbarumin I (1). White wax; mp 42–44°C; $[\alpha]_D$=+28.0° (*c* 1.0 mg/mL, EtOH); UV (EtOH) λ_{max} (log ϵ) 204 (3.58) nm; CD (EtOH) $\Delta\epsilon$ (nm) 2.70×10^4 (205), -1.85×10^4 (228); IR ν_{max} (KBr) 3390, 2957, 2926, 1721, 1191, 1046 cm^{-1}; ^1H and ^{13}C NMR (Table 2); EIMS *m/z* 228 $[M]^+$ (3), 210 (12), 200 (10), 157 (10), 143 (30), 125 (85), 95 (100), 79 (90), 57 (37), 41 (26). HREIMS *m/z* 228.1364 (calcd for $C_{12}H_{20}O_4$, 228.1361).

Herbarumin II (2). Pale yellow needles; mp 100–101°C; $[\alpha]_D$=+30.8° (*c* 1.0 mg/mL, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (3.14) nm; CD (MeOH) $\Delta\epsilon$ (nm) 5.31×10^4 (207), -2.37×10^4 (222); IR ν_{max} (KBr) 3560, 2962, 2931, 2873, 1720, 1053 cm^{-1}; ^1H and ^{13}C NMR (Table 3); EIMS *m/z* 244 $[M]^+$ (3), 226 (5), 208 (3), 143 (75), 97 (43), 67 (100), 57 (39), 41 (29). HREIMS *m/z* 244.1316 (calcd for $C_{12}H_{20}O_5$, 244.1310).

Acetylation of herbarumin II (2). A solution of **2** (10 mg) in pyridine (0.1 mL) and Ac_2O (0.1 mL) was kept at room temperature for 48 h, diluted with CH_2Cl_2 (20 mL), washed with HCl 1 N (3×20 mL), saturated $NaHCO_3$ solution (3×20 mL) and water (3×20 mL), dried over anhydrous Na_2SO_4 and evaporated to dryness, affording the triacetate **4** (12.3 mg) as pale yellow needles; mp 63–65°C; IR ν_{max} (KBr) 1749, 1720, 1646, 1618, 1220; ^1H and ^{13}C NMR (Table 3); EIMS *m/z* 370 $[M]^+$ (3), 228 (15), 327 (3), 310 (10), 284 (10), 267 (20), 225 (70), 183 (90), 95 (100), 67 (54), 43 (33); FABMS (positive) *m/z* 371 $[M+H]^+$ (80), 311 (95), 269 (20), 181 (23), 137 (100), 136 (75), 107 (22), 43 (42).

Benzoylation of herbarumins I (1) and II (2). To a stirred solution of **1** or **2** (5.0 mg) and 4-bromobenzoyl chloride (Sigma) (10.0 mg), in pyridine (2.5 mL) was added 4-(dimethylamino)pyridine (0.5 mg). The reaction mixture was stirred at room temperature for 48 h, diluted with CH_2Cl_2 (10 mL), washed with HCl 1 N (3×10 mL), saturated $NaHCO_3$ solution (3×10 mL) and water (3×10 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness. In each case, the resulting mixture was purified by TLC on silica gel (CH_2Cl_2) to yield 3.8 mg **3** and 4.0 mg of **6**. Compound **3**: white needles; UV (EtOH) λ_{max} (log ϵ) 245 (3.96), 204 (4.01); CD (EtOH) $\Delta\epsilon$ (nm) -4.75×10^5 (211), -6.42×10^4 (237), 9.26×10^5 (255); IR ν_{max} (KBr) 3560, 2962, 2931, 2873, 1720, 1053 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.00 (2H, d, *J*=8.0 Hz, H-2′, H-6′ Bz), 7.90 (2H, d, *J*=8.0 Hz, H-2′, H-6′ Bz), 7.68 (2H, d, *J*=8.0 Hz, H-3′, H-5′ Bz), 7.60 (2H, d, *J*=8.0 Hz, H-3′, H-5′ Bz), 5.95 (1H, t, *J*=2.0 Hz, H-7), 5.75 (1H, dd, *J*=15.9, 2.0 Hz, H-6), 5.50 (1H, dddd, *J*=16.8, 10.0, 4.0, 2.0 Hz, H-5), 5.37 (1H, ddd, *J*=12.0, 9.3, 2.7 Hz, H-9), 5.03 (1H, dd, *J*=9.3, 2.0 Hz, H-8), 0.92 (3H, t, *J*=7.5 Hz, H-13); FABMS (positive) *m/z* 595 $[M+H]^+$ (8), 549 (8), 522 (6), 413 (6), 391 (20), 307 (12), 257 (5), 183 (100), 154 (90), 136 (70), 55 (42), 43 (25), 23 (8). Compound **6**: white needles; UV (MeOH) λ_{max} (log ϵ) 245 (4.42), 204 (4.08); CD (MeOH) $\Delta\epsilon$ (nm) -1.89×10^6 (221), 3.71×10^5 (255); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.05 (2H, d, *J*=8.0 Hz, H-2′, H-6′ Bz), 7.93 (2H, d, *J*=8.0 Hz, H-2′, H-6′ Bz), 7.75 (2H, d, *J*=8.0 Hz, H-2′, H-6′ Bz), 7.63 (2H, d, *J*=8.0 Hz, H-3′, H-5′ Bz), 7.58 (2H, d, *J*=8.0 Hz, H-3′, H-5′ Bz), 7.56 (2H, d, *J*=8.0 Hz, H-3′, H-5′ Bz), 6.08 (1H, t, *J*=2.0 Hz, H-7), 5.81 (1H, dd, *J*=16.8, 2.0, H-6), 5.70 (1H, ddd, *J*=10.0, 5.4, 6.3, H-9), 5.60 (1H, dddd, *J*=16.8, 10.0, 4.0, 2.0 Hz, H-5), 5.33 (1H, dd, *J*=10.0, 2.0 Hz, H-8), 5.09 (1H, dd, *J*=9.6, 3.9 Hz, H-2), 0.86 (3H, t, *J*=7.5, H-13); FABMS (positive) *m/z* 794 $[M+H]^+$ (6), 705 (8), 593 (18), 413 (10), 391 (20), 389 (8), 307 (18), 289 (12), 257 (8), 183 (100), 154 (96), 136 (78), 55 (43), 43 (20), 23 (15).

Preparation of 7,8-*O*,*O*′-isopropylideneherbarumin II (5). Herbarumin II (**2**) (10.0 mg) in dry Me_2CO (7.0 mL) was stirred with dry $CuSO_4$ (70.0 mg) under reflux for 12 h. The mixture was filtered and evaporated under reduced pressure to give an oily residue. The residue was purified by HPLC on a silica gel column [8.2 mL min^{-1}, hexane–*i*-PrOH–MeOH, (80:10:10)] to yield **5** (8.0 mg): yellow oil, ^1H NMR ($CDCl_3$, 300 MHz) δ 5.60 (1H, ddd, *J*=16.5, 9.2, 5.4 Hz, H-5), 5.38 (1H, dd, *J*=16.5, 7.5 Hz, H-6), 5.11 (1H, ddd, *J*=10.3, 9.3, 2.5 Hz, H-9), 4.78 (1H, dd, *J*=7.5, 6.0 Hz, H-7), 4.27 (1H, t, *J*=3.5 Hz, H-2), 3.89 (1H, dd, *J*=10.3, 6.0 Hz, H-8), 1.46 (3H, s, CH_3), 1.36 (3H, s, CH_3), 0.91 (3H, t, *J*=7.5 Hz). HREIMS *m/z* 284.1629 (calcd for $C_{15}H_{24}O_5$, 284.1623).

Molecular modeling calculations

Minimum energy structures were generated using the MMX

force field as implemented in the PCMODEL program V 6.00 (Molecular Modeling Software available from Serena Software, Box 3076, Bloomington, IN 47402–3076). The conformational search for the ten-membered ring of **1** was carried out considering the pertinent restrictions according to the vicinal coupling constants found in the C(6)–C(7)–C(8)–C(9)–C(10) fragment. For the remaining portion of the ten-membered ring, those conformations obtained by torsion angle rotations of ca. 30° were minimized and evaluated taking into account the E_{MMX} convergence criterion. The propyl side-chain was minimized considering all possible alternate conformations.

Phytogrowth-inhibitory bioassays

The phytogrowth-inhibitory activity of the extract and pure compounds was evaluated on seeds of *A. hypochondriacus* using a Petri dish bioassay.[13] In addition, a direct bioautographic bioassay system was employed to guide secondary fractionation and speed up the isolation of active compounds. The direct bioautographic assay was carried out as previously described. The results were analyzed by ANOVA ($p<0.05$), and IC_{50} (50% inhibitory concentration) values expressed in molar concentration were calculated by Probit analysis based on percent of radicle growth inhibition. The extract was evaluated at 1, 10, 100 and 1000 $\mu g\ mL^{-1}$. The pure compounds were tested at 0.1, 1, 10 and 100 $\mu g\ mL^{-1}$. 2,4-D (2,4-dichlorophenoxyacetic acid) was used as the positive control. The bioassays were performed at 28°C.

Acknowledgements

This work was supported by grants from CONACYT (27978N), DGAPA (IN207300) and PADEP (207347 and 108329). We thank Isabel Chávez, Luis Velasco-Ibarra, Javier Pérez-Flores, Rocío Patiño, Georgina Duarte and Oscar Yañez from Instituto de Química and Facultad de Química, UNAM, for recording some NMR, MS, UV, IR, and CD spectra. The technical support of Laura Acevedo, Perla Castañeda and Rebeca Martínez-Flores is also acknowledged. J. F. Rivero-Cruz acknowledges the fellowships awarded by DGAPA-UNAM and CONACYT to carry out graduate studies. The authors are indebted to Drs José Calderón and Carlos Céspedes for their valuable support at some stages of the present investigation.

References

1. Domsh, K. H.; Gams, W.; Anderson, T. H. *Compendium of Soil Fungi*, Academic: London, 1980; Vol. 1.
2. Pedras, M. S. C.; Abrams, S. R.; Séguin-Swartz, G.; Quail, J. W.; Jia, Z. *J. Am. Chem. Soc.* **1989**, *111*, 1904–1905.
3. Ichihara, A.; Oikawa, H.; Hayashi, K.; Sakamura, S.; Furusaki, A.; Matsumoto, T. *J. Am. Chem. Soc.* **1983**, *105*, 2907–2908.
4. Parisi, A.; Piattelli, M.; Tringali, C.; Di San Lio, G. M. *Phytochemistry* **1993**, *32*, 865–867.
5. Kobayashi, A. T.; Kono, Y.; Yamaguchi, G. O. *Pestic. Sci.* **1999**, *55*, 602–604.
6. Evidente, A.; Lanzetta, R.; Capasso, R.; Andolfi, A.; Vurro, M.; Zonno, M. C. *Phytochemistry* **1995**, *40*, 1637–1641.
7. Evidente, A.; Lanzetta, R.; Capasso, R.; Andolfi, A.; Vurro, M.; Zonno, M. C. *Phytochemistry* **1997**, *44*, 1041–1045.
8. Brebaum, S. N.; Borland, G. J. *Plant Disease* **1999**, *83*, 200.
9. MacGahren, W. J.; Ellestad, G. A.; Morton, G. O.; Kunstmann, M. P. *J. Org. Chem.* **1972**, *37*, 1636–1639.
10. Chandler, I. M.; McIntyre, C. R.; Simpson, T. J. *Perkin Trans. 1* **1992**, 2271–2284.
11. Chandler, I. M.; McIntyre, C. R.; Simpson, T. J. *Perkin Trans. 1* **1992**, 2285–2293.
12. Pinkerton, F.; Strobel, G. A. *Proc. Natl. Acad. Sci., USA* **1982**, *73*, 4007–4011.
13. Mata, R.; Macías, M. R.; Rojas, S.; Lotina-Hennsen, B.; Toscano, R.; Anaya, A. *Phytochemistry* **1998**, *49*, 441–449.
14. Evidente, A.; Lanzetta, R.; Capasso, R.; Vurro, M.; Bottalico, A. *Phytochemistry* **1993**, *34*, 999–1003.
15. Burket, U.; Allinger, N. L. *Molecular Mechanics*, ACS Monograph, American Chemical Society: Washington, DC, 1982; Vol. 177.
16. Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783–2792.
17. Cerda-García-Rojas, C. M.; Zepeda, L. G.; Joseph-Nathan, P. *Tetrahedron Comp. Methodol.* **1990**, *3*, 113–118.
18. Hilderbrandt, R. L.; Wieser, J. D.; Montgomery, L. K. *J. Am. Chem. Soc.* **1973**, *95*, 8598–8605.
19. Harada, N.; Nakanishi, K. *Acct. Chem. Res.* **1972**, *5*, 257–263.
20. Lin, Y. Y.; Risk, M.; Ray, S. M.; Van Engen, D.; Clardy, J.; Golik, J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773–6775.
21. Evidente, A.; Capasso, R.; Andolfi, A.; Vurro, M.; Zonno, M. C. *Natural Toxins* **1998**, *6*, 183–188.