# Stagonolides G−I and Modiolide A, Nonenolides Produced by *Stagonospora cirsii*, a Potential Mycoherbicide for *Cirsium arvense*

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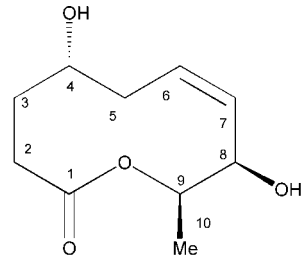
*Stagonospora cirsii* Davis, a fungal pathogen isolated from *Cirsium arvense* (commonly called Canada thistle) and proposed as a potential mycoherbicide of this perennial noxious weed, produces phytotoxic metabolites in liquid and solid cultures. Stagonolide, the main phytotoxic metabolite, and five new related nonenolides, named stagonolides B−F, were isolated from the fungus. When grown on solid culture, nonenolide yields increased. A further four nonenolides were isolated and characterized by spectroscopy. Three were new compounds and named stagonolides G−I, and the fourth was identified as modiolide A, previously isolated from *Paraphaeosphaeria* sp., a fungus separated from the horse mussel. Leaf disk-puncture assays at 1 mg/mL of stagonolides H−I and modiolide A were phytotoxic to *C. arvense*. Only stagonolide H inhibited chicory seedling root growth. The most potent toxin, stagonolide H, indicated selectivity when tested on leaves of eight different plants: Canada thistle was most sensitive to the compound.

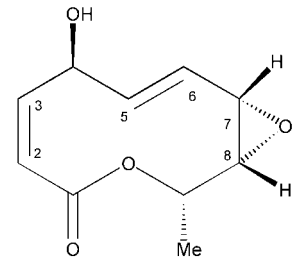
The perennial weed *Cirsium arvense* (L.) Scop. (commonly called Canada thistle) is a problem on arable land in North America, New Zealand, and Europe. Since it can propagate from cut pieces of underground shoots, the plant is not affected by mechanical weed control.[1] Because of restrictions in applying of chemical herbicides,[2] extensive research has been carried out worldwide to find new effective and ecologically friendly alternatives. A possible solution is the use of weed pathogens for biological control. However, these are living organisms, and their efficacy is often questionable because of environmental conditions.[3] Some biocontrol agents may produce phytotoxic metabolites (phytotoxins), and these, or their analogues, could potentially be used for the development of new selective herbicides.[4−6] Recently, some fungi were proposed by our group as mycoherbicides for *C. arvense*, and all of them have shown to produce phytotoxins with potential practical application. This is the case of *Phyllosticta cirsii*, which was shown to produce new oxazatricyalkalenones and a new pentasubstituted bicyclo-octatrienyl acetic acid ester named phyllostictines A−D[7] and phyllostoxin,[8] as well as *Phoma exigua* var. *exigua*, which synthesized *p*-hydroxybenzaldehyde, the cytochalasins B, F, Z2, and Z3, and deoxaphomin.[9]

The pycnidial fungus *Stagonospora cirsii* Davis is a foliar pathogen of *C. arvense*, for which the development of a mycoherbicide was demonstrated.[10] In a preliminary study, it was found that the fungus was capable of producing phytotoxins, because culture filtrates were phytotoxic to leaves and roots of the thistle.[11] The main phytotoxic metabolite produced by *S. cirsii* in liquid culture, named stagonolide, was isolated and characterized as a new nonenolide.[12] Recently, the same fungus grown on solid culture showed an increased capacity to produce nonenolides. Five new stagonolides, named stagonolide B−F, were isolated and chemically characterized, including their biological properties.[13]

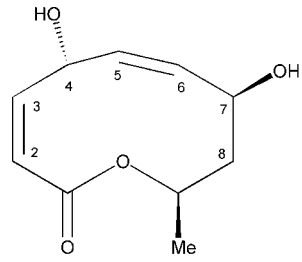
Herein we describe the isolation, structural elucidation, and biological activity of four nonenolides produced in solid culture by *S. cirsii*. The three compounds were named stagonolides G−I (**1**−**3**, respectively). Their structures were determined by NMR and MS techniques. We also identified modiolide A (**4**), previously isolated from *Paraphaeosphaeria* sp., a fungus separated from the horse mussel *Modiolus auriculatus*.[14]



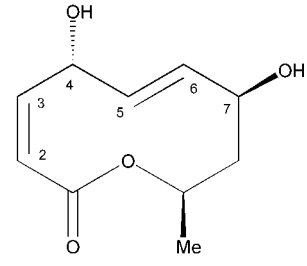

Stagonolide G, **1**




Stagonolide H, **2**




Stagonolide I, **3**




Modiolide A, **4**

## Results and Discussion

The solid culture of *S. cirsii* (1 kg) was exhaustively extracted with acetone−water. The organic extract, exhibiting high phytotoxicity, was purified by a combination of column chromatography and TLC, yielding four metabolites. Compound **2** was obtained as a solid, and compounds **1**, **3**, and **4** were obtained as homogeneous oils. Preliminary $^{1}H$ and $^{13}C$ NMR investigations showed that all metabolites were nonenolides.

Further investigation (essentially 1D and 2D NMR and MS techniques) showed that one of these nonenolides had the same structure as modiolide A (**4**). In particular, the NMR data previously recorded in $CDCl_3$ (see Tables 1 and 2) differed from those reported in $CD_3OD$ because of solvent differences.[14] Modiolide A was previously isolated in conjunction with the analogous modiolide B. These were the first two 10-membered macrolides isolated from

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**Table 1.** $^1$H NMR Data for Stagonolides G−I (**1**−**3**) and Modiolide A (**4**)[a]

| | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| position | $\delta_H$ ($J$ in Hz) | $\delta_H$ ($J$ in Hz) | $\delta_H$ ($J$ in Hz) | $\delta_H$ ($J$ in Hz |
| 2 | 2.56 (2H) m | 5.93 d (12.0) | 5.68 dd (11.8, 2.1) | 5.89 d (12.5) |
| 3 | 2.35 m | 6.11 dd (12.0,6.4) | 6.31 dd (11.8, 4.0) | 5.91 d (12.5) |
| | 1.95 ddd (17.7, 10.9, 9.5) | | | |
| 4 | 4.54 m | 4.76 br dd (6.4, 2.2) | 5.71 br ddd (9.6, 4.0, 2.1) | 4.79 br d (8.7) |
| 5 | 2.63 ddd (14.5, 7.7, 7.7) | 5.96 dd (15.9, 2.2) | 5.40 dd (10.3, 9.6) | 5.74 dd (15.9, 8.7) |
| | 2.48 ddd (14.5, 5.2, 5.2) | | | |
| 6 | 5.67 ddd (11.1, 7.7, 5,2) | 5.88 dd (15.9, 1.6) | 5.50 dd (10.3, 8.2) | 5.63 dd (15.9, 10.4) |
| 7 | 5.60 dd (11.1, 8.2) | 3.65 dd (4.3, 1.6) | 4.97 br ddd (9.8, 8.2, 3.0) | 4.26 br dd (10.4, 11.1, 3.2)) |
| 8 | 4.11 dd (8.2, 8.2) | 2.94 br d (4.3, <1.0) | 2.24 ddd (13.9, 9.8, 7.0) | 1.92 ddd (12.9, 3.2, 1.2) |
| | | | 1.87 ddd (13.9, 3.9, 3.0) | 1.81 ddd (12.9,11.1, 10.8) |
| 9 | 3.67 dq (8.2, 6.5) | 5.43 br q (6.9, <1.0) | 5.11 m | 5.31 ddq (10.8, 6.3, 1.2) |
| 10 | 1.15 d (6.5) | 1.50 d (6.9) | 1.41 d (6.5) | 1.28 d (6.3) |
| OH | 5.67 br s | 4.85 br s | 5.88 br s | 1.67 br s |
| OH | 3.64 s | | 5.72 br s | 1.56 br s |

[a] The chemical shifts are in $\delta$ values (ppm) from TMS. 2D $^1$H,$^1$H (COSY) and $^{13}$C,$^1$H (HSQC) NMR experiments delineated the correlations of all protons and the corresponding carbons. The assignments are in agreement with the values reported for stagonolides B−F,[13] modiolides,[14] and herbarumins.[22]

**Table 2.** $^{13}$C NMR Data for Stagonolides G−I (**1**−**3**) and Modiolide A (**4**)[a]

| | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| position | $\delta_C$ mult.[b] | $\delta_C$ mult.[b] | $\delta_C$ mult.[b] | $\delta_C$ mult.[b] |
| 1 | 178.0 qC | 167.7 qC | 164.8 qC | 167.0 qC |
| 2 | 28.7 $CH_2$ | 126.1 CH | 121.0 CH | 122.8 CH |
| 3 | 27.5 $CH_2$ | 133.9 CH | 149.1 CH | 136.1 CH |
| 4 | 79.6 CH | 66.9 CH | 66.8 CH | 71.3 CH |
| 5 | 33.7 $CH_2$ | 131.3 CH | 129.4 CH | 131.0 CH |
| 6 | 127.8 CH | 119.7 CH | 134.5 CH | 137.3 CH |
| 7 | 132.5 CH | 55.8 CH | 64.5 CH | 72.1 CH |
| 8 | 72.3 CH | 56.3 CH | 42.6 $CH_2$ | 42.7 $CH_2$ |
| 9 | 70.8 CH | 65.6 CH | 68.4 CH | 68.7 CH |
| 10 | 18.7 $CH_3$ | 18.6 $CH_3$ | 20.8 $CH_3$ | 21.3 $CH_3$ |

[a] The chemical shifts are in $\delta$ values (ppm) from TMS. 2D $^1$H,$^1$H (COSY, TOCSY) and $^{13}$C,$^1$H (HSQC) NMR experiments delineated the correlations of all protons and the corresponding carbons. The assignments are in agreement with the values reported for stagonolides B−F,[13] modiolides,[14] and herbarumins.[22] [b] Multiplicities determined by DEPT spectrum.

**Table 3.** HMBC Data for Stagonolides G−I (**1**−**3**) and Modiolide A (**4**)

| C | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| 1 | $H_2$-2 | H-3, H-2 | H-3, H-2 | H-2 |
| 2 | | H-4, H-3 | H-4 | |
| 3 | $H_2$-2 | H-4, H-2 | H-5, H-2 | |
| 4 | $H_2$-5, H-3′ | H-6, $H_2$-3 | H-6, H-5 | H-6, H-3, H-2 |
| 5 | H-7, H-3′ | H-7, H-6, H-4, H-3 | H-7, H-6, H-4 | H-7, H-6, H-3 |
| 6 | H-8, $H_2$-5, | H-7, H-5, H-4 | H-7, H-5 | H-5, H-4 |
| 7 | $H_2$-5 | H-8, H-6, H-5 | $H_2$-8, H-5 | $H_2$-8, H-5 |
| 8 | H-7 | H-7, H-6, Me-10 | Me-10, H-7 | Me-10, H-6 |
| 9 | H-8, Me-10 | H-8, Me-10 | $H_2$-8 | Me-10, H-8′ |
| 10 | | H-9, H-8 | H-9, H-8 | |

the culture broth of a fungus separated from the horse mussel *Modiolus auriculatus*, collected at Hedo Cape, Okinawa Island, which was identified as *Paraphaeosphaeria* sp. The antibacterial and antifungal activities of modiolides A and B against *Micrococcus luteus* (MIC value 16.7 mg/mL) and *Neurospora crassa* (MIC value 33.3 mg/mL), respectively, were also reported.[14] The first total synthesis of modiolide A, based on the whole-cell yeast-catalyzed asymmetric reduction of a propargyl ketone, was recently described.[15]

The three new nonenolides were structurally similar to stagonolide and stagonolides B−F, previously isolated from the culture filtrates of the same fungus and, consequently, were named stagonolides G−I (**1**−**3**). All three new stagonolides lacked an *n*-propyl group at C-9, present in stagonolide and stagonolide B, the *n*-propyl group being substituted in each case by a methyl group, as in stagonolides C−F.

Stagonolide G (**1**) had a molecular formula of $C_{10}H_{16}O_4$ as deduced from HRESIMS data, consistent with three degrees of unsaturation, two of which resulted from a double bond and a lactone group as deduced from the IR spectrum and preliminary $^1$H and $^{13}$C NMR investigations. The IR spectrum also showed bands attributable to hydroxy groups,[16] while the UV spectrum had no absorption maxima. Inspection of the $^1$H NMR spectrum (Table 1) showed the presence of a doublet of double doublets ($J$ = 11.1, 7.7, and 5.2 Hz) and a double doublet ($J$ = 11.1 and 8.2 Hz) at $\delta$ 5.67 and 5.60, typical of two protons (H-6 and H-7, respectively) of a *cis*-1,2-disubstituted olefinic group,[17,18] and signals of two oxymethine carbons (H-4 and H-8) resonating as a very complex multiplet and a double doublet ($J$ = 8.2 and 8.2 Hz) at $\delta$ 4.54 and 4.11, respectively. H-4 coupled in the COSY spectrum[19] with two doublets of double doublets ($J$ = 14.5, 7.7, 7.7 and 14.5, 5.2, 5.2 Hz) of $CH_2$-5 at $\delta$ 2.63 and 2.48 and with a multiplet and a doublet of double doublets ($J$ = 17.7, 10.9, and 9.5 Hz) of $CH_2$-3 at $\delta$ 2.35 and 1.95, respectively. H-8, in turn, coupled with H-7 and with a double quartet ($J$ = 8.2 and 6.5 Hz) of H-9 at $\delta$ 3.67. The $^{13}$C NMR spectrum (Table 2) showed the signals typical of a lactone carbonyl, two secondary olefinic, and three oxymethine carbons at $\delta$ 178.0, 132.5, and 127.8 and 79.6, 72.3, and 70.8, which, from the couplings observed in the HSQC spectrum,[19] were attributed to C-1, C-7 and C-6, and C-4, C-8, and C-9, respectively.[17] The couplings observed in the same spectrum also allowed assignment of the resonances observed in the $^{13}$C NMR spectrum at $\delta$ 33.7, 28.7, 27.5, and 18.7 to C-5, C-2, C-3, and C-10.[20]

The coupling observed in the COSY and HSQC spectra allowed assignment of the chemical shifts to all protons and corresponding carbons (Tables 1 and 2, respectively) and for stagonolide G the structure of 4,8-dihydroxy-9-methyl-6-nonen-9-olide (**1**).

This structure was supported by the sodium cluster observed in the HRESIMS spectrum at *m*/*z* 223.2355 and by the couplings observed in the HMBC spectrum[19] (Table 3).

Stagonolide H (**2**) had a molecular formula of $C_{10}H_{12}O_4$ as deduced from the HRESIMS spectrum, consistent with five degrees of unsaturation, three of which were the same as in **1**. The IR spectrum showed bands attributable to a hydroxy group,[16] while the UV spectrum had no absorption maxima. Both $^1$H and $^{13}$C NMR spectra showed that **2** compared with **1** differed by the presence of a C-5−C-6 double bond instead of C-6−C-7, an additional C-2−C-3 double bond, and a C-7−C-8 epoxy group as in stagonolide D. In fact, the $^1$H NMR spectrum (Table 1) showed the presence of two double doublets ($J$ = 15.9 and 2.2 Hz and $J$ = 15.9 and 1.6 Hz), a doublet ($J$ = 12.0 Hz), and a double doublet ($J$ = 12.0 and 6.4 Hz) at $\delta$ 5.96 and 5.88 and at $\delta$ 5.93 and 6.11,

**Table 4.** 2D $^1$H-NOE (NOESY) Data Obtained for Stagonolides G−I (**1**−**3**) and Modiolide A (**4**)

| **1** | | **2** | | **3** | | **4** | |
|---|---|---|---|---|---|---|---|
| considered | effects | considered | effects | considered | effects | considered | effects |
| H-9 | Me-10, H-8, H-7 | H-9 | Me-10, H-8 | H-9 | Me-10, $H_2$-8 | H-9 | H-8, Me-10 |
| H-8 | Me-10, H-9, H-7, $H_2$-5 | H-8 | H-9, H-7 | H-7 | H-8′ | H-8 | H-9, H-7, Me-10 |
| H-7 | H-9, H-8, | H-7 | H-8, H-6 | H-6 | H-8′ | H-7 | H-8 |
| H-6 | $H_2$-5, H-4 | H-4 | H-5, H-3 | H-3 | H-2 | H-6 | H-7, H-8, H-4 |
| $H_2$-5 | H-8, H-6, H-4, $H_2$-3 | | | | | H-5 | H-7, H-8, H-4 |
| H-4 | H-6, $H_2$-5, $H_2$-3 | | | | | H-4 | H-3 |
| | | | | | | Me-10 | H-9, H-8 |

typical of two protons (H-5 and H-6) of a *trans*-disubstituted double bond and two protons (H-2 and H-3) of a *cis*-disubstituted olefinic group.[17,18] Furthermore, a double doublet ($J$ = 4.3 and 1.6 Hz) and a broad doublet ($J$ = 4.3 and <1.0 Hz), which were assigned to H-7 and H-8, were observed at $\delta$ 3.65 and 2.94, respectively, typical chemical shift values for a 1,2-disubstituted oxirane ring.[17,21] As expected, in the COSY spectrum H-7 coupled with the double doublet of the adjacent olefinic proton (H-6), while H-8 coupled with the broad quartet ($J$ = 6.9 and <1.0 Hz) of H-9 at $\delta$ 5.43. H-3 coupled with H-4 ($\delta$ 4.76, br d, $J$ = 6.4 and 2.2 Hz) oxymethine that also coupled with the olefinic adjacent H-5. The $^{13}$C NMR spectrum (Table 2) showed, in addition to the lactone carbonyl resonance at $\delta$ 167.7 (C-1), the signals typical of four olefinic carbons ($\delta$ 133.9, 131.3, 126.1, and 119.7), two oxymethine carbons ($\delta$ 66.9 and 65.6), and an epoxy ring, ($\delta$ 56.3 and 55.8). These resonances could be assigned via the HSQC spectrum to C-3, C-5, C-2, and C-6, and C-4 and C-9, and C-8 and C-7, respectively. The same technique also allowed the assignment of the resonance at $\delta$ 18.6 to C-10.[20]

The coupling observed in the COSY and HSQC spectra permitted the assignment of chemical shifts to all protons and corresponding carbons (Tables 1 and 2, respectively), and to stagonolide H the structure 7,8-epoxy-4-hydroxy-9-methyl-2,5-nonadien-9-olide. This structure was supported by the sodium clusters observed in the HRESIMS spectrum at *m*/*z* 219.2056 and by the couplings observed in the HMBC[19] spectrum (Table 3).

Stagonolide I (**3**) had a molecular formula of $C_{10}H_{14}O_4$ as deduced from the HRESIMS spectrum, which was consistent with four degrees of unsaturation, being the same as **2**, as deduced from the IR spectrum and preliminary $^1$H and $^{13}$C NMR results. The IR spectrum showed also bands attributable to the hydroxy group,[16] while the UV spectrum had no absorption maxima. Examination of both the $^1$H and $^{13}$C NMR spectra showed that **3**, in comparison to **2**, differed by the absence of the C-7−C-8 epoxy group in **2**, the *cis*-configuration of the C-5−C-6 double bond, and the presence of a second oxymethine carbon (C-7). In fact, in the $^1$H NMR spectrum, H-7 appeared as a broad doublet of double doublets ($J$ = 9.8, 8.2, and 3.0 Hz) at $\delta$ 4.97, which in the COSY spectrum coupled with H-6 ($\delta$ 5.50, dd, $J$ = 10.3 and 8.2 Hz) and with the protons of $CH_2$-8 ($\delta$ 2.24, dd, $J$ = 13.9, 9.8, and 7.0 Hz and $\delta$ 1.87, dd, $J$ = 13.9, 3.9 and 3.0), respectively. The latter, in turn, coupled with the multiplet of H-9 at $\delta$ 5.11. Furthermore, the olefinic H-5 resonated as a double doublet ($J$ = 10.3 and 9.6 Hz) at $\delta$ 5.40, being also coupled with H-4 ($\delta$ 5.71, br dd, $J$ = 9.6, 4.0, and 2.1 Hz), which was also coupled with the adjacent olefinic proton H-3 ($\delta$ 6.31, dd, $J$ = 11.8 and 4.0 Hz), and this, in turn, coupled with H-2 ($\delta$ 5.68, dd, $J$ = 11.8 and 2.1 Hz). The $^{13}$C NMR spectrum (Table 2) showed, apart from the expected signal of the lactone carbonyl at $\delta$ 164.8 (C-1), resonances typical of four olefinic carbons ($\delta$ 149.1, 134.5, 129.4, and 121.0) as well as those of three oxymethine carbons ($\delta$ 68.4, 66.8, and 64.5). These resonances could be assigned via HSQC spectrum to C-3, C-6, C-5, and C-2, and C-9, C-4, and C-7, respectively. The couplings observed in the same spectrum also allowed the assignment of the signals observed in the $^{13}$C NMR spectrum at $\delta$ 42.6 and 20.8 to C-8 and C-10, respectively.[20]

The coupling observed in the COSY and HSQC spectra confirmed the chemical shifts of all protons and corresponding carbons (Tables 1 and 2, respectively) and permitted assignment of the structure of stagonolide I as 4,7-dihydroxy-9-methyl-2,5-nonadien-9-olide. This was supported by the sodium cluster observed in the HRESIMS spectrum at *m*/*z* 221.2117 and by the couplings observed in the HMBC spectrum (Table 3).

The relative configuration of the epoxy functionality in stagonolide H (**2**) was assigned by comparison of the $^3J_{H,H}$ spin systems with the data reported for suitable 1,2-disubstituted *cis*- and *trans*-oxirans.[17,21] The configuration of the double bonds of all nonenolides was determined comparing the coupling constants of the olefinic protons to the reported values.[17]

The relative configuration of the stereogenic carbons of stagonolides G−I (**1**−**3**) was essentially determined by comparison of the $^3J_{H,H}$ spin systems with those of modiolide A and herbarumin I, the absolute configuration of which was independently determined by CD, using a suitable derivative, and NOESY and molecular mechanics modeling, respectively.[14,22] Previously, assignment of the relative configuration of the stereogenic carbons of stagonolide B−F was done comparing their NMR data with those of herbarumin I and putaminoxin.[13] In particular, in stagonolide G (**1**), H-8 is α ($J_{7,8} = J_{8,9}$ = 8.2 Hz), as is H-7 in modiolide A ($J_{6,7}$ = 7.5 and $J_{7,8}$ = 11.4 Hz), H-9 is α ($J_{8,9}$ = 8.2 Hz), as in herbarumin I ($J_{8,9}$ = 9.8 Hz), and H-4 should be $\beta$, as no effect was observed in the NOESY spectrum between this proton and H-8 and H-9, being both α. In stagonolide H (**2**), H-4 is α ($J_{3,4}$ = 6.4 and $J_{4,5}$ = 2.2 Hz), while in modiolide A H-4 is $\beta$ ($J_{3,4}$ = 3.5 and $J_{4,5}$ = 7.3 Hz); H-7 and H-8 both are $\beta$ ($J_{6,7}$ = 1.6 and $J_{8,9}$ < 1.0 Hz), as in stagonolide D ($J_{6,7}$ = 4.8 and $J_{7,8}$=3.9 and $J_{8,9}$ = 2.6 Hz); H-9 is $\beta$ ($J_{8,9}$ < 1.0 Hz), while in herbarumin I H-9 is α ($J_{8,9}$ = 9.8 and 2.5 Hz). Finally, in stagonolide I (**3**), H-4 is $\beta$ ($J_{3,4}$ = 4.0 and $J_{4,5}$ = 9.6 Hz), as in modiolide A ($J_{3,4}$ = 3.5 and $J_{4,5}$ = 7.3 Hz), H-7 is α ($J_{6,7}$ = 8.2 and $J_{7,8}$ = 9.8 Hz), as in modiolide A ($J_{6,7}$ = 7.5 and $J_{7,8}$ = 11.4 Hz), and H-9 is α ($J_{8,9}$ = 7.0 and $J_{8,9'}$ = 3.9 Hz), as in modiolide A ($J_{8,9}$ = 11.4 and $J_{8,9'}$ = 2.5 Hz).

The relative configuration assigned to stagonolides G−I (**1**−**3**) is in agreement with the NOE effects observed in the NOESY spectra[16] (Table 4). In fact, a significant NOE effect was observed in stagonolides G and H between H-8 and H-9 and between H-7 and H-8, respectively.

Nonenolides **1**−**4** tested at 1 mg/mL were shown to have different phytotoxic activities. Stagonolide H was the most toxic to the leaves of *C. arvense*, stagonolide I and modiolide A were significantly less active, and stagonolide G was inactive (Figure 1). The minimum concentration of stagonolide H causing leaf lesions in *C. arvense* was about 30 $\mu$g/mL (~1.5 × 10$^{-4}$ M) (Figure 2). It is similar to the level of activity of stagonolide.[12]

At 1 mg/mL only stagonolide H inhibited root growth in chicory seedlings (85% compared to control), while other compounds were inactive at the concentration used. Stagonolide H appeared to have less inhibitory activity to chicory seedlings than stagonolide, which showed similar activity at 1 $\mu$g/mL.[12]

Leaves of eight plant species were found to have different sensitivities to stagonolide H (**2**, Figure 3). Leaves of *C. arvense* were significantly more sensitive to **2** (necrotic lesion diameter ~7.5

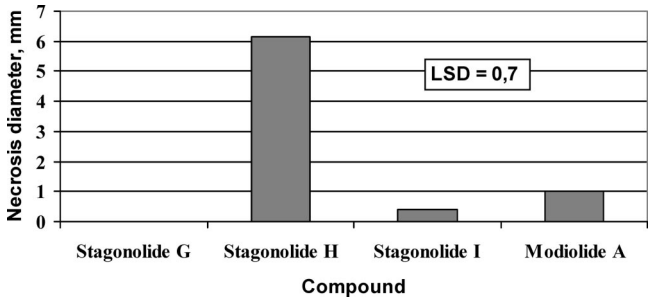


**Figure 1.** Phytotoxicity of nonenolides **1**−**4** at 1 mg/mL in the *C. arvense* leaf disk-puncture bioassay (48 h post-application). The concentration of MeOH was 2% v/v, which is nontoxic to leaves of *C. arvense* plants in the control.

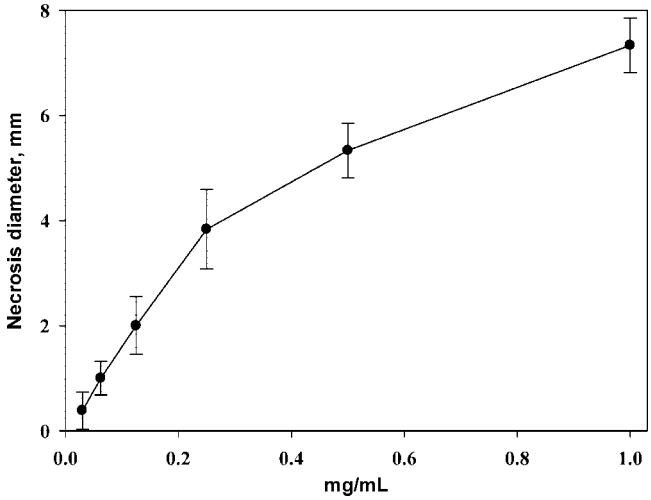


**Figure 2.** Dose−response relationship for stagonolide H by the *C. arvense* leaf disk-puncture bioassay (48 h post-application). Bars indicate standard deviation.

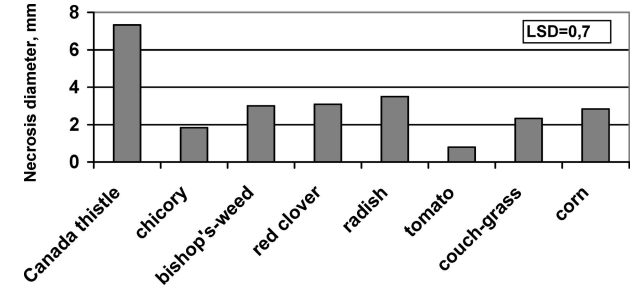


**Figure 3.** Effect of stagonolide H at 1 mg/mL on a range of various plant species using a leaf disk-puncture assay (72 h post-application). The concentration of MeOH was 2% v/v, which is nontoxic to leaves of all plants in the control.

mm, 72 h post-application) than other plants tested (necrotic lesion diameter <4 mm). Tomato leaves were slightly sensitive to the toxin (Figure 3). Stagonolide H showed both high phytotoxicity and selectively, and this phytotoxin may be considered a potential natural herbicide.

Macrolides, and particularly nonenolides, are common naturally occurring compounds. Structurally close nonenolides appear to be the putaminoxins and the herbarumins, phytotoxins with potential herbicidal activity that were isolated from *Phoma putaminum*[23–25] and *P. herbarum*,[22,26] respectively. Other phytotoxins are the pinolidoxins and aspinolides A−C isolated from *Ascochyta pinodes*[27,28] and *A. ochraceus*,[29] respectively. In addition, the structurally close nonendien-9-olides are modiolide and fusanolide, isolated from *Paraphaeosphaeria* sp.[14,15] and *Fusarium* sp.,[30] respectively.

## Experimental Section

**General Experimental Procedures.** Optical rotation was measured in $CHCl_3$ on a Jasco P-1010 digital polarimeter; IR spectra were recorded as neat on a Perkin-Elmer Spectrum One FT-IR spectrometer, and UV spectra were taken in MeCN solution on a Perkin-Elmer Lambda 25 UV/vis spectrophotometer. $^1H$ and $^{13}C$ NMR spectra were recorded at 600 and 150 and 75 MHz, respectively, in $CDCl_3$ on Bruker spectrometers. The internal standard was $CDCl_3$. Carbon multiplicities were determined by DEPT spectra.[19] DEPT, COSY-45, HSQC, HMBC, and NOESY experiments[19] were performed using Bruker microprograms. ESI and HRESIMS spectra were recorded on a Micromass Q-TOF Micro instrument. Analytical and preparative TLC were performed on Si gel (Merck, Kieselgel 60 F254, 0.25 and 0.50 mm, respectively) or reversed-phase (Whatman, KC18 F254, 0.20 mm) plates; the spots were visualized by exposure to UV light and/or by spraying first with 10% $H_2SO_4$ in MeOH and then with 5% phosphomolybdic acid in EtOH, followed by heating at 110 °C for 10 min. CC: Si gel (Merck, Kieselgel 60, 0.063−0.200 mm).

**Fungal Strain.** *Stagonospora cirsii* was originally isolated from diseased leaves of *Cirsium arvense* (L.) Scop. and was maintained in the Culture Collection of the All-Russian Institute of Plant Protection, Saint-Petersburg, Russia (No. C-163). Before use the fungus was stored on potato-sucrose medium at 5 °C.

**Production, Extraction, and Purification of Stagonolides G−I (1−3) and Modiolide A (4).** The fungus was grown on autoclaved millet in 1000 mL Erlenmeyer flasks (millet 100 g, $H_2O$ 60 mL) for 14 days in the dark. Fungal metabolites were extracted from the dry mycelium according to the published protocol,[13] which was slightly modified. The dried material (1000 g) was extracted with acetone−water (1:1). After evaporation of acetone, NaCl (300 g/L) was added to the aqueous residue, and the latter was extracted with EtOAc. The organic extracts were combined, dried ($Na_2SO_4$), and evaporated in vacuo to yield a brown, oily residue.

The organic extract (1 g) obtained from the culture (1 kg), showing high phytotoxicity, was purified by Si gel column chromatography eluted with $CHCl_3$−*i*-PrOH (9:1), to give 13 groups of homogeneous fractions. The residues of the second and third fractions were combined (353 mg) and further purified by column chromatography, eluted with EtOAc−*n*-hexane (65:35), yielding 13 further groups of homogeneous fractions. Fractions were tested for bioactivity against *C. arvense* as described below, and those showing phytotoxicity were further purified. The residue (65 mg) of the fourth fraction was purified by preparative TLC on Si gel, eluting with petroleum ether−acetone (7:3), to yield a crystalline solid (**2**, $R_f$ 0.27; 18.6 mg) named stagonolide H. The residue (69.8 mg) of the 10th fraction from the initial column was purified by preparative TLC on silica gel, using $CHCl_3$−*i*-PrOH (88:12), to yield six bands. The fifth band ($R_f$ 0.27; 27.0 mg) was further purified by preparative TLC on Si gel, eluted with EtOAc−MeOH (96:4), yielding two homogeneous oily compounds: one was named stagonolide G (**1**, $R_f$ 0.45; 1.5 mg), and the other was indentified as modiolide A (**4**, $R_f$ 0.52; 12 mg). The residue (30.4 mg) of the 11th fraction of the initial column was purified by preparative TLC on Si gel, using $CHCl_3$−*i*-PrOH (88:12) for elution, to yield five bands. The fourth band ($R_f$ 028; 10.6 mg) was further purified by preparative TLC on Si gel, eluted with EtOAc−MeOH (96:4), yielding a homogeneous oily compound ($R_f$ 0.48; 2.0 mg), named stagonolide I (**3**).

**Stagonolide G (1), (5α,9*β*,10*β*)-5,9-dihydroxy-10-methyl-3,4,5,6,9,10-hexahydro-oxecin-2-one:** colorless oil; $[\alpha]^{25}_D$ +96 (*c* 0.1); UV $\lambda_{max}$ < 200 nm; IR $\lambda_{max}$ 3388, 1765, 1727, 1259 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectra, see Tables 1 and 2; HRESIMS (+) *m/z* 223.2355 $[M + Na]^+$ (calcd for $C_{10}H_{16}NaO_4$, 223.2264).

**Stagonolide H (2), (1α,2α,7*β*,10α)-7-hydroxy-2-methyl-3,11-dioxa-bicyclo[8.1.0]undeca-5,8-dien-4-one:** white crystalline solid; $[\alpha]^{25}_D$ +12 (*c* 0.2); UV $\lambda_{max}$ < 200 nm; IR $\lambda_{max}$ 3426, 1723, 1635, 1239 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectra, see Tables 1 and 2; HRESIMS (+) *m/z* 219.2056 $[M + Na]^+$ (calcd for $C_{10}H_{12}NaO_4$, 219.1945).

**Stagonolide I (3), (5α,8*β*,10*β*)-5,8-dihydroxy-10-methyl-5,8,9,10-tetrahydro-oxecin-2-one:** colorless oil; $[\alpha]^{25}_D$ +50 (*c* 0.2); UV $\lambda_{max}$ < 200 nm; IR $\lambda_{max}$ 3278, 1713, 1640, 1217 $cm^{-1}$; $^1H$ and $^{13}C$ NMR

spectra, see Tables 1 and 2; HRESIMS (+) *m/z* 221.2117 $[M + Na]^+$ (calcd for $C_{10}H_{14}NaO_4$, 221.2105).

**Modiolide A:** colorless oil; $[\alpha]^{25}_D$ +38 (*c* 0.2, MeOH); UV MeOH $\lambda_{max}$ 204 nm ($\epsilon$ 6400); IR $\lambda_{max}$ 3436, 1713, 1280 $cm^{-1}$ (lit. 14 $[\alpha]^{25}_D$ +42 (*c* 0.25, MeOH); UV (MeOH) $\lambda_{max}$ (log) 204 nm ($\epsilon$ 6400); $^1H$ and $^{13}C$ NMR spectra, see Tables 1 and 2; HRESIMS (+) *m/z* 205.0852 $[M + Na]^+$ (calcd for $C_{10}H_{14}NaO_3$, 205.0841; lit. 11: EIMS *m/z* 180 $(M - H_2O)^+$ and 198 $(M)^+$; HREIMS *m/z* 198.0892 ($M^+$, calcd for $C_{10}H_{14}O4$, 198.0891).

**Leaf Disk-Puncture Assays.** Culture filtrates, their organic extract, the chromatographic fractions, and pure compounds **1**−**4** were assayed at 1 mg/mL by leaf disk-puncture bioassay on *C. arvense*. The disks were obtained from *C. arvense* leaves and punctured as previously reported.[12] Crude organic extracts, chromatographic fractions, and pure compounds were dissolved in a small amount of MeOH and then brought up to the desired concentration with distilled $H_2O$. Additionally, a spectrum of phytoxicity of **2** was evaluated at 1 mg/mL on a number of plant species using a leaf disk-puncture bioassay: *Chicorium intybus* L. (chicory), *Aegopodium podagraria* L. (bishop's weed), *Trifolium pretense* L. (red clover), *Raphanus sativus* L. (radish), *Solanum lycopersicum* L. (tomato), *Elytrigia repens* (L.) Desv. ex Nevski (couch-grass), and *Zea mays* L. (corn). The plants were produced from seeds in a greenhouse, and the discs were obtained as previously reported.[12] The concentration of MeOH was 2% v/v, which is nontoxic to leaves of all plants in the control. Droplets (10 $\mu$L) of the test solution were applied on the disks and then incubated for two days in the conditions previously reported.[12] The diameter of the necrotic lesions was measured.

**Seedlings Bioassay.** Seedlings of chicory with rootlets of 1−2 mm length were soaked for 1 h in a 1 mg/mL solution of compound **1**−**4** (concentration of MeOH 2%) and then incubated on a moistened Petri dish as previously reported.[12] The length of rootlets in treatment was compared with the control (2% MeOH).

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