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Herbicidal Potential of Stagonolide, a New Phytotoxic Nonenolide from *Stagonospora cirsii*

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Stagonospora cirsii is a pathogen of *Cirsium arvense*, causing necrotic lesions on leaves of this noxious weed. The fungus produced toxic metabolites when grown in liquid culture. A new phytotoxin, named stagonolide, was isolated and characterized as (8*R*,9*R*)-8-hydroxy-7-oxo-9-propyl-5-nonen-9-olide by spectroscopic methods. Stagonolide was shown to be a nonhost-specific but selective phytotoxin. Leaves of *C. arvense* were most sensitive and leaves of tomato and pepper (both *Solanaceae*) were less sensitive to stagonolide, which was assayed at 5×10^{-3} M, than other plants. Stagonolide assayed at 5×10^{-6} M was demonstrated to be a strong inhibitor of root growth in seedlings of *C. arvense* and some other *Asteraceae* species. Seedlings growth in wheat and radish was much less affected by the toxin, and seedlings of cucumber were insensitive to it.

KEYWORDS: ***Cirsium arvense*; Asteraceae; *Stagonospora cirsii*; mycoherbicide; phytotoxin; nonenolide; stagonolide**

INTRODUCTION

The perennial weed, *Cirsium arvense* (L.) Scop., is a problem weed through arable land in North America, New Zealand, and Europe, including the European part of the Russian Federation. This "ideal" weed propagates both vegetatively, by lateral roots, and generatively, by seeds. It is resistant to common mechanical weed control methods (*1, 2*). Considering some restriction in application of chemical herbicides, extensive research work has been carried out in many countries worldwide to find new effective and ecologically friendly alternatives. A possible solution is the use of weed pathogens for biological control. However, they are living organisms, and their efficacy is often not stable because it strongly depends upon environmental conditions (*3*). Some of the biocontrol agents can produce phytotoxic metabolites (phytotoxins) as pathogenicity factors. The phytotoxins or their analogues could be used for the development of new selective herbicides (*4*).

One attempt to realize the mentioned approach was to isolate a phytotoxin produced by *Septoria cirsii* Niessl, a foliar pathogen of *C. arvense*. It was identified as β-nitropropionic acid (*5*). However, this toxin displayed a broad activity spectrum, and its production was found previously in some other microorganisms (*5, 6*).

Stagonospora cirsii Davis is another foliar pathogen of *C. arvense*. The biological potential of this pycnidial fungus for the development of a mycoherbicide was demonstrated (*7*). In a preliminary study, it was found that a strain of *S. cirsii* was capable of producing phytotoxins because culture filtrates of this fungus demonstrated phytotoxic activity to leaves and roots of *C. arvense* (*8*). The present paper describes the isolation and chemical and biological characterization of a toxin produced by *S. cirsii*, named stagonolide, which is a new nonenolide.

MATERIALS AND METHODS

Fungal Strain, Culture Medium, and Growth Conditions. For stagonolide (**1**), production of a monoconidial strain of *S. cirsii* C-163 was used. It was originally isolated from diseased leaves of *C. arvense* (L.) Scop. and was maintained in the culture collection of the All-Russian Institute of Plant Protection, St. Petersburg, Russia. Before use, the fungus was stored in tubes on a potato-sucrose medium at 5 °C. For the production of toxic metabolites, two mycelial blocks (5 mm in diameter) cut from colony edges were added to 2 L Roux-type culture flasks containing 300 mL of modified Czapek media (*8*). The cultures were incubated under static conditions in the dark for 2 weeks, then filtered, and assayed for phytotoxic activity.

General Experimental Procedures. Melting point determination was performed on a PTP-M apparatus (Khimlabpribor, Klin, Russia). The infrared (IR) spectrum was recorded in $CHCl_3$ on a Specord 75IR spectrometer, and the ultraviolet (UV) spectrum was recorded in EtOH on a Beckman Coulter DU 800 spectrometer. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-500 spectrophotometer in $CDCl_3$ at 500 and/or 125.6 MHz. The same solvent was used as an internal standard. Mass spectra (ESI–TOF) were taken on a MX 5303 spectrometer (Institute of Analytical Industry, St. Petersburg, Russia). Preparative chromatographic separation was performed on silica gel using Merck 60. The solvent systems were (A) *n*-hexane, (B) *n*-hexane–Et_2O (9:1), (C) *n*-hexane–Et_2O (8:2), (D) *n*-hexane–Et_2O (7:3), (E) *n*-hexane–Et_2O (6:4), (F) *n*-hexane–Et_2O (1:1), (G) *n*-hexane–EtOAc (1:1), and (H) EtOAc.

Production, Extraction, and Purification of Stagonolide. The original culture filtrate (3 L) was acidified with 1 M orthophosphoric acid to pH 2–3 and further extracted in a separating funnel with EtOAc (3× 1 L). Organic extracts were combined, dried out with Na_2SO_4, and evaporated by a rotary evaporator at a temperature of no greater

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than 40 °C. The resulting brown dry residue was fractionated by the column with silica gel under gradient elution in solvent systems: A–H, 50 mL each. Only fraction E demonstrated the phytotoxic activity. After solvent evaporation, there was about 120 mg of an uncolored crystalline substance, named stagonolide **1**, whose purity was proven by high-performance liquid chromatography (HPLC).

Stagonolide (1). mp: 70–72 °C. UV λ_{max} (log ε) nm: 228 (4.07). IR ν_{max}, cm^{-1}: 3000–3500 (OH), 2900–3000 (CH_2, CH_3), 1740 (lactone C═O), 1700 (ketonic C═O), 1640 (C═C), 1190 (O–CO). ^{1}H NMR: 6.42 (1H, d, J = 16.0 Hz, H-6), 6.31 (1H, m, H-5), 4.65 (1H, t, J = 9.5, 9.5 Hz, H-9), 4.04 (1H, dd, J = 9.5, 6.1 Hz, H-8), 3.57 (1H, d, J = 6.1 Hz, OH), 2.50 (1H, m, H-4a), 2.44 (1H, dd, J = 14.4, 9.5 Hz, H-2a), 2.13 (1H, dd, J = 14.4, 6.2 Hz, H-2b), 2.05 (1H, m, H-4b), 2.03 (1H, m, H-3a), 1.96 (1H, m, H-10b), 1.92 (1H, m, H-3b), 1.65 (1H, m, H-10a), 1.42 (1H, m, H-11b), 1.31 (1H, m, H-11a), 0.91 (3H, t, J = 7.2, H-12). ^{13}C NMR: 199.6 (C-7, C═O), 174.2 (C-1, C(O)═O), 143.1 (C-5), 131.9 (C-6), 76.5 (C-8), 74.5 (C-9), 34.2 (C-10), 34.0 (C-2), 33.5 (C-4), 25.0 (C-3), 18.0 (C-11), 13.7 (C-12). ESI–TOF MS m/z (relative intensity): 227.1275 ($C_{12}H_{19}O_4$, calcd 227.1283) $[M + H]^+$ (37), 210 $[M + H-OH]^+$ (15), 209.1172 ($C_{12}H_{17}O_3$, calcd 209.1178) $[M + H-H_2O]^+$ (100), 192 $[M + H-OH-C_2H_4]^+$ (12), 191 $[M + H-H_2O-C_2H_4]^+$ (73), 184 $[M + H-C_3H_7]^+$ (21), 164 $[M + H-C_2H_4-CO]^+$ (4), 163 $[M + H-OH-C_2H_4-CO]^+$ (21).

$NaBH_4$ Reduction of Stagonolide. Sodium boron hydride (1.9 mg, 0.05 mmol) was added to a solution of **1** (22.6 mg, 0.1 mmol) in EtOH (3 mL) within 5 min under intensive stirring. The resulted mixture was further stirred for 2 h at room temperature. Reaction products were monitored with thin-layer chromatography (TLC) (solvent system F). After evaporation of EtOH, a reduction product was diluted in water (10 mL) and isolated by Et_2O extraction of the aqueous suspension (3 times, 2:1). The combined organic layer was washed with saturated sodium chloride solution. The solvents were then removed to yield **2** (21.0 mg, 92%) in pure form. IR ν_{max}, cm^{-1}: 3000–3500, 2850–3000, 1715, 1635, 1200, 1055, 985. ^{1}H NMR ($CDCl_3$, 500 MHz): 5.59 (1H, d, J = 16.0 Hz), 5.50 (1H, dddd, J = 16.0, 9.8, 3.9, 2.4 Hz), 4.93 (1H, td, J = 9.5, 2.6 Hz), 4.41 (1H, m), 3.49 (1H, dd, J = 9.5, 2.3 Hz), 2.40 (1H, br s), 2.39 (1H, br d, J = 12.4 Hz), 2.30 (1H, ddd, J = 14.0, 6.0, 2.4 Hz), 2.14 (1H, br s), 2.00 (1H, dd, J = 14.0, 13.0 Hz), 1.94 (1H, m), 1.87 (2H, m), 1.72 (1H, m), 1.56 (1H, m), 1.37 (1H, m), 1.29 (1H, m), 0.90 (3H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$,125.6 MHz): 176.3, 130.6, 124.9, 73.7, 73.3, 70.2, 34.4, 33.7, 33.3, 24.7, 18.0, 13.8. ESI–TOF MS m/z (relative intensity): 229.1444 ($C_{12}H_{21}O_4$, calcd 229.1439) $[M + H]^+$ (100), 211 $[M + H-H_2O]^+$ (43), 193 $[M + H-2H_2O]^+$ (13).

Leaf Disc-Puncture Bioassay. Culture filtrates, their chromatographic fractions, and pure stagonolide were assayed on the host plant using a leaf disc-puncture assay. The discs were cut of well-expanded leaves of *C. arvense*, placed in a moistened chamber, and punctured in the center with a sharp needle. Both the fractions and the pure toxin were first dissolved in a small amount of EtOH and then brought up to the desired concentration with distilled water (final concentration of EtOH was 20%). The toxin was tested at concentrations from 1×10^{-5} to 1×10^{-2} M. To evaluate selectivity of stagonolide, it was also tested (at 5×10^{-3} M) on a number of nonhost plants. The plants were produced from seeds in greenhouse conditions, and the discs were cut from young well-expanded true leaves. Droplets (10 μL) of the test solution were applied on the discs and then incubated for 2 days under artificial light (16 h day/8 h night) and at a controlled temperature (24 °C day/20 °C night). The diameter of necrotic lesions was measured.

Seedlings Bioassay. Seedlings of *C. arvense* with rootlets of 1–2 mm length were soaked for 1 h in a stagonolide solution of different concentrations (from 1×10^{-7} to 1×10^{-2} M in 5% EtOH) and then incubated on moistened filter paper in Petri dishes for 48 h in darkness at 24 °C. The length of rootlets in treatments was compared with the control (5% EtOH). Selectivity of the toxin at a concentration of 1 μg/mL was evaluated additionally on seedlings of several cultivated plants and *C. arvense*.

Antifungal Activity. Antifungal activity of the toxin was assayed on *Candida tropicalis* grown on a peptone–glucose agar at 27 °C, according to the method previously described (*9*). The toxin was tested at 100 μg/disc.

Antibiotic Activity. Antibacterial activity was tested on *Escherichia coli* and *Bacillus subtillis*, according to the method described (*9*). The toxin was tested at 50 μg/disc on 24 h colonies of the test organisms produced on bacterial nutrient agar at 27 °C.

Zootoxic Activity. The stagonolide was assayed on the infusorium, *Colpoda steinii*, by the official technique for the determination of toxicity of animal food, which is currently used in the Russian Federation (*10*). The standard Lozina–Lozinskogo media (2 mL) was added to the dried infusorium culture containing about 5000 cells/mL, and then, the resulted suspension was incubated for 24 h at 25 °C before use. The toxin solution in 4% EtOH was added to the infusorium (1:1, v/v) to its final concentration from 5×10^{-5} to 4×10^{-4} M. After a course of incubation (from 3 to 180 min), the number of immobile cells (%) was counted. In the control treatment, *C. steinii* culture was prepared in 4% EtOH. If 100% of the infusoria cells become immobile within 3 min of exposure with the toxin, the studied substance demonstrates strong toxicity, and if they loose activity in ≥180 min, the substance should be evaluated as low toxic.

Data Analysis. Means were compared using Student's t test or by Fisher's LSD coefficients. Biological assays were carried out in duplicate and were reproducible.

RESULTS

Chemical Identification. Phytotoxic culture filtrates of *S. cirsii* were extracted with EtOAc, giving an oily brown residue. After its purification by CC, as described in detail in the Materials and Methods, a homogeneous crystalline compound withstanding recrystalization was obtained and named stagonolide (**1**).

Data of mass spectrometric analysis showed that the isolated toxin had the molecular formula $C_{12}H_{18}O_4$. Its infrared spectrum contains bands for the presence of hydroxyl, ester and ketonic carbonyl, and olefinic groups. The UV spectrum showed an absorption maximum for a double bond conjugated with a carbonyl group. This fact was also confirmed by ^{1}H and ^{13}C NMR spectra. The significant downfield shift observed for the proton signals of the double bond (δ 6.42 and 6.31 for H-6 and H-5), when compared with the proton signals for the isolated double bond (δ 5.30 and 5.52) of putaminoxin (*11*) and the shift of the carbon signal C-5 (δ 143.1), showed its conjugation with a carbonyl group. Further, the proton signal H-6 at δ 6.42 resonated as a doublet (J = 16.0 Hz), while the other olefinic proton (H-5) appeared as a multiplet at δ 6.31, showing a trans configuration of the double bond. Furthermore, the ^{13}C NMR spectrum contained two signals, which were assigned to carbonyl groups: the first signal corresponded to the ester carbonyl group (δ 174.2), and the second signal corresponded to the carbon of the α,β-unsaturated ketonic group (δ 199.6), which is in agreement with the IR spectrum data.

The ^{1}H–^{1}H correlation spectroscopy (COSY) contained two groups of cross-peaks. Each group demonstrated sequential couplings of protons. For the first group, ^{1}H–^{1}H COSY showed the coupling between the proton H-5 and the protons of the methylene group CH_2-4, resonating as complex multiplet signals at δ 2.05 and 2.50. In turn, the protons of the latter correlated with the protons of the methylene group CH_2-3, resonating as multiplets at δ 1.92 and 2.03. Finally, the protons of the group CH_2-3 coupled with the protons of the CH_2-2 methylene group appearing as two doublets of doublets at δ 2.13 (J = 14.4 and 6.2 Hz) and δ 2.44 (J = 14.4 and 5.8 Hz), a typical chemical-shift value of proton α-located to a carbonyl group (*12*). These values are in agreement with published data (*11*) as well.

The analysis of the second group of the proton signals in the ^{1}H–^{1}H COSY spectrum established the couplings for three

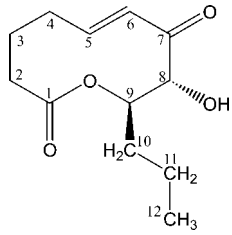

Figure 1. Structure of stagonolide isolated from the liquid culture of *S. cirsii*.

protons of the terminal methyl group (Me-12) of a propyl side chain observed as a triplet ($J = 7.2$ Hz) at δ 0.91 and the protons of the adjacent methylene group (CH_2-11), resonating as two complex multiplets at δ 1.31 and 1.42. The latter protons also coupled with the protons of another methylene group (CH_2-10), appearing as two multiplets at δ 1.65 and 1.96. The propyl side chain was bonded to the oxygenated tertiary C-9 carbon based on the coupling between the protons of H_2C-10 and H-9, with the latter being observed as a double triplet ($J = 9.5$ and 9.5 Hz) at δ 4.65. A significant downfield shift of this proton and the corresponding carbon (δ 74.5) shows that C-9 is the closing center for the lactone ring (**Figure 1**). Literature data on the structural analysis of the compounds with similar structure (*11*, *13*, *14*) supported our observations. For instance, the proton bonded to C-9 in putaminoxin (*11*) also produces the multiplet signal at δ 5.00 (NMR spectrum recorded in $CDCl_3$).

These results were supported by a further investigation of the ^{1}H–^{1}H COSY spectrum, which showed the coupling between H-9, with the proton of a secondary hydroxylated carbon (HC-8) appearing as a double doublet ($J = 9.5$ and 6.1 Hz), and also being coupled with the proton of the geminal hydroxyl group, resonating as a doublet at δ 3.57. Consequently, HC-8 should be bonded to the carbonyl group, O═C-7.

For determination of the coupling order for two groups of the studied atom, a ^{13}C–^{1}H NMR (COLOC) experiment was carried out. The corresponding spectrum showed the coupling of the carbon atom of the carbonyl group at δ 174.2 (O═C-1) with the group CH_2-10 of the propyl side chain and with the CH-9 group via an oxygen bridge. The coupling of the carbon atom of the carbonyl group at δ 199.6 (O═C-7) with the group of CH-8 [J (^{13}C-7–^{1}H-8) = 2 Hz] and with the CH_2-6 group [J (^{13}C-7–^{1}H-6) = 6 Hz)] was also observed. These results are not in contradiction with the data mentioned above.

This structure of phytotoxin was further supported by mass spectrometry data. The ESI–MS showed sets of fragmentation peaks typical for macrolides (*15*). Pseudomolecular ion peak at m/z 227.1275 $[M + H]^+$ lost in succession H_2O, C_2H_4, and CO or OH, C_2H_4, and CO producing the ions at m/z 209 (the main peak), 191, and 163 or 210, 192, and 164, respectively. An alternative fragmentation path was the loss of the propyl group by the pseudomolecular ion $[M + H]^+$ that produced in the ion at m/z 184.

Reduction of **1** with $NaBH_4$ in EtOH gave a substance with spectral data similar to herbarumin I (**2**) known as (7*S*,8*S*,9*R*)-7,8-dihydroxy-9-propyl-5-nonen-9-olide (*16*). A theoretically possible stereoisomer of **2** was not found that can be connected with the orienting action of a neighbor hydroxyl group. According to literature data at mentioned conditions of the reaction only, a carbonyl group of aldehydes or ketones can be reduced (*17*) and, at the same time, inversion of substituents at C-8 and C-9 was impossible. Therefore, the stereochemical structure of stagonolide is similar to herbarumin I.

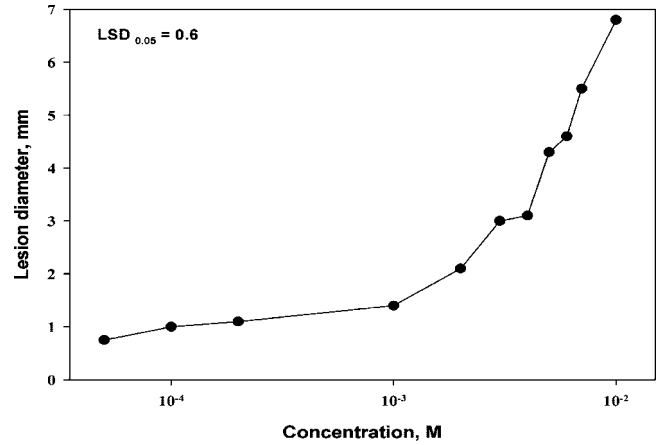

Figure 2. Dose–response relationship for stagonolide by the *C. arvense* leaf disc-puncture bioassay.

Additionally, the nuclear Overhauser effect spectrometry (NOESY) experiment showed the nuclear Overhauser effect (NOE) between both H-8 and H-6 and H-8 and CH_2-10. This situation is possible only for (8*R*,9*R*) and (8*R*,9*S*) isomers of stagonolide. A comparative analysis of J (H-8–H-9) and values of dihedral angles between bonds C8-H and C9-H, which was based on the Karplus relations (in Bothner-By's modification), showed that stagonolide should be formulated as (8*R*,9*R*)-8-hydroxy-7-oxo-9-propyl-5-nonen-9-olide (**1**) (**Figure 1**).

Biological Assays. The first symptoms (necrotic spots) appeared about 10 h post application of stagonolide on punctured leaf discs of *C. arvense*. The minimal concentration of the toxin caused the symptoms to be about 1×10^{-4} M (**Figure 2**). When the toxin was used at a concentration of 5×10^{-3} M, necrotic spots reached ~4 mm in diameter 48 h after treatment. Assayed at concentration of 5×10^{-3} M on leaf discs of weed species and cultivated plants, stagonolide did not demonstrate specificity to *Asteraceae* representatives. Besides *C. arvense*, leaves of hollyhock, sunflower, lettuce, sow-thistle, radish, and peppermint were highly sensitive to the toxin. However, two species of *Solanaceae* tested were insensitive to it (**Table 1**).

Stagonolide inhibited the growth of seedling roots of *C. arvense* at concentrations $\geq 1 \times 10^{-6}$ M and decreased their length on more than 30% compared with the control (**Figure 3**). Assayed at a concentration of 1 μg/mL ($\sim 5 \times 10^{-6}$ M) on seedlings of different plat species, stagonolide was shown to be more selective to *Asteraceae* species (about 70% inhibition). Root growth of wheat and radish seedlings was less affected by stagonolide (about 30% inhibition). Cucumber seedlings were insensitive to stagonolide at this concentration (**Figure 4**).

The toxin of *S. cirsii* was low toxic for *Colpoda steinii* (Protozoa) at concentrations $\geq 2 \times 10^{-4}$ M and nontoxic at concentrations $\leq 1 \times 10^{-4}$ M (**Table 2**). The symptoms of the toxicity appeared as a gradual deceleration of the infusoria motion and changing the cell form of infusorium to globular, with a increase in the sizes. At the concentration of 50–100 μg of stagonolide/disc, the toxin of *S. cirsii* showed weak antimicrobial activity against the fungus *C. tropicalis*, with both $Gram^+$ and $Gram^-$ bacteria tested.

DISCUSSION

Stagonolide is structurally related to several known fungal nonenolides, for instance, putaminoxins (*11*, *13*, *14*) isolated from *Phoma putaminum*, a pathogen of *Erigeron annuus* (*Asteraceae*), pinolidoxins (*18*, *19*) isolated from *Ascochyta pinodes*, a pathogen of peas (*Fabaceae*), and herbarumins

Table 1. Effect of Stagonolide on a Range of Various Plant Species Using a Leaf Disc-Puncture Assay

plant species			
plant family	common name	latin name	necrosis of leaves (mm)[a]
Asteraceae	Canada thistle	*Cirsium arvense*	3.3
	lettuce	*Lactuca sativa*	2.8
	zinnia	*Zinnia elegans*	1.0
	sow-thistle	*Sonchus arvensis*	2.2
	sunflower	*Helianthus annuus*	3.0
Solanaceae	pepper	*Capsicum annuum*	0.3
	tomato	*Lycopersicon esculentum*	0.2
Lamiaceae	peppermint	*Mentha piperita*	2.8
Brassicaceae	radish	*Raphanus sativus*	2.2
Malvaceae	hollyhock	*Alcea rugosa*	3.0
Fabaceae	pea	*Pisum sativum*	2.3
Poaceae	wheat	*Triticum aestivum*	2.0
$LSD_{0.05}$			1.1

[a] Droplets (10 μL) of toxin solution (5×10^{-3} M) were applied on previously needle-punctured leaves. The effect was observed 2 days after droplet application.

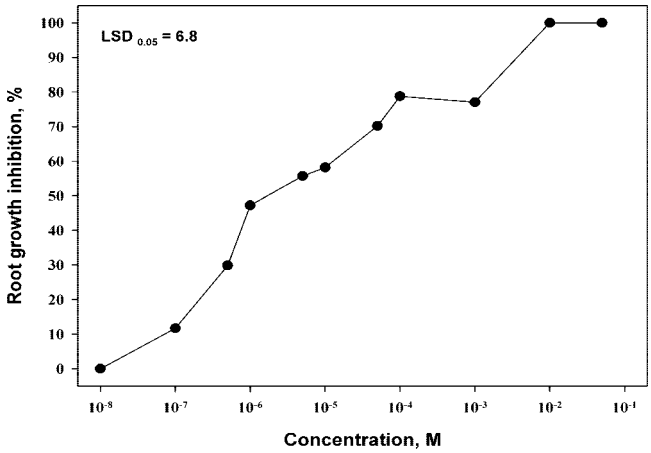

Figure 3. Dose–response relationship for stagonolide by the *C. arvense* seedlings bioassay.

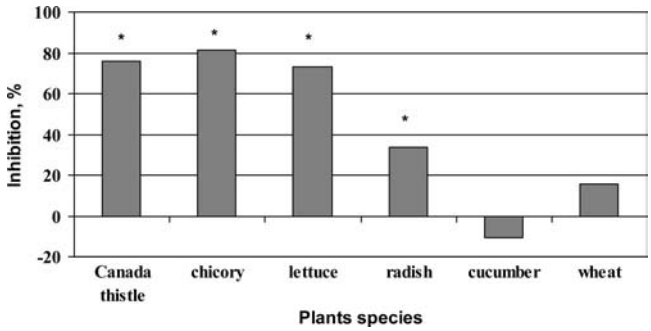

Figure 4. Inhibitory effect of stagonolide on the root growth of seedlings of various plant species.

isolated from *Phoma herbarum*, a pathogen of *Zea mays* (*Poaceae*) (*16*, *20*). Among them, herbarumin I is the most related to stagonolide. Surprisingly, among investigated natural phytotoxic nonenolides or their chemical analogues, there was no structure with ketonic carbonyl group in the lactone ring at C-7, similar to stagonolide (*16*, *20*–*22*).

Obviously, phytotoxic nonenolides are common for *Phoma* and *Ascochyta* plant pathogens. The taxonomic position of *S. cirsii* strains is now under revision, and our preliminary results showed that it belongs to the genus *Phoma*, section *Heterospora* (Berestetskiy, unpublished results). Therefore, screening biologically active nonenolides in *Phoma*- and *Ascochyta*-like fungi looks prospective. *S. cirsii* C-163 was found to produce a large amount of stagonolide (~40 mg/L) on modified Czapek liquid media compared to the production of herbarumin I (~0.4 mg/L) by *P. herbarum* on M-1-D liquid media (*16*). Therefore, *S. cirsii* can be considered a good producer of the phytotoxic metabolite.

Table 2. Zootoxicity of Stagonolide, Number of Immobile Cells of *C. steinii* (%)

	time of incubation of the test organism in toxin solution (min)						
toxin concentration (M, $\times 10^{-4}$)	3	10	30	45	60	90	180
4	0	0	33.8		73.5	100	100
2	0	0	29.2	50.0	61.8	93.8	94.1
1	0	0	0	21.7	20.0	18.0	0
0.5	0	0	0		10	5.0	0
0	0	0	0	0	0	0	4
$LSD_{0.05}$			2.3	2.6	2.5	2.7	2.6

Assayed on leaves of different plant species, both stagonolide and putaminoxin were shown not to be host-specific toxins. However, *C. arvense* and *E. annuus* demonstrated higher sensitivity to respective toxins than other plants (*11*). Interestingly, tomato leaves were less sensitive to both pinolidoxin and stagonolide than leaves of plants from other families (*23*). The nature of selectivity of stagonolide to *Solanaceae* representatives should be further studied.

Herbarumin I and stagonolide demonstrated high potency as root growth inhibitors at low concentrations ($IC_{50} \sim 5 \times 10^{-5}$ M) for *Amaranthus hypochodriacus* L. (*16*) and *C. arvense* ($IC_{50} \sim 5 \times 10^{-6}$ M), respectively. At this concentration, stagonolide is not zootoxic (**Table 2**). Moreover, stagonolide seems to be a selective phytotoxin against *Asteraceae* species when tested by the seedling bioassay (**Figure 4**). The specificity of the herbarumins was not studied. Because the main task in the control of perennial weeds is the prevention of the growth of lateral roots, stagonolide is interesting for the development of a natural herbicide against *C. arvense*.

CONCLUSION

The investigations described above enabled us to determine the structure for phytotoxin **1** as (8*R*,9*R*)-8-hydroxi-7-oxo-9-propyl-5-nonen-9-olide, a new compound belonging to the nonenolide group having strong inhibitory activity on root growth.

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