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Agropyrenol, a Phytotoxic Fungal Metabolite, and Its Derivatives: A Structure−Activity Relationship Study

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ABSTRACT: Agropyrenol is a phytotoxic substituted salicylic aldehyde produced in liquid culture by *Ascochyta agropyrina* var. *nana*, a potential mycoherbicide proposed for the control of the perennial weed *Elytrigia repens*. In this study, six derivatives obtained by chemical modifications of the toxin were assayed for phytotoxic, antimicrobial, and zootoxic activities, and the structure−activity relationships were examined. Each compound was tested on non-host weedy and agrarian plants, fungi, Gram-positive and Gram-negative bacteria, and brine shrimp larvae. The results provide insights into the structure−activity relationships of agropyrenol. Both the double bond and the diol system of the 3,4-dihydroxypentenyl side chain as well as the aldehyde group at C-1 of the phenolic ring of agropyrenol proved to be important for the phytotoxicity. The lesser polar 3′,4′-O,O′-isopropylidene of agropyrenol also showed significant zootoxic and slight antimicrobial activities. This finding could be useful in devising new natural herbicides for practical application in agriculture.

KEYWORDS: *Ascochyta agropyrina* var. *nana*, weeds, *Elytrigia repens*, phytotoxins, agropyrenol, SAR

■ INTRODUCTION

Bioactive fungal metabolites have long been considered for their potential direct use as novel agrochemicals or as a lead for new natural pesticides or to discover novel mechanisms of action.[1,2] More specifically, fungal toxins and their derivatives have also been considered as sources of novel and safe natural herbicides.[3−5] Having this perspective, the fungus *Ascochyta agropyrina* (Fairman) Trotter var. *nana* Punith., isolated from diseased leaves of the perennial weed *Elytrigia repens* (L.) Desv. ex Nevski (quack grass), was recently studied and proven to be a good source of novel metabolites. Indeed, from the solid culture of *A. agropyrina*, papyracillic acid was first isolated, a fungal metabolite already described for its antimicrobial, nematicidal, and cytotoxic activities[6] but not yet for its interesting phytotoxicity.[7] Later, some key derivatives were prepared by chemical modifications of the metabolite, and their phytotoxic and antimicrobial activities were assayed against a number of crop and weedy plants, fungi, bacteria, and nematodes. The results obtained allowed for identification of some structural features important for the biological activity but also to show that one of the three monoacetyl derivatives was very promising and could be further considered for its herbicidal activity.[7]

Interestingly, when *A. agropyrina* was grown in liquid culture, it produced metabolites other than papyracillic acid, the main of which was identified as a new 6-monosubstituted salicylic aldehyde, named agropyrenol (1; Figure 1). Together with agropyrenol, two other novel metabolites were isolated, i.e., a trisubstituted naphthalene carbaldehyde and a pentasubstituted
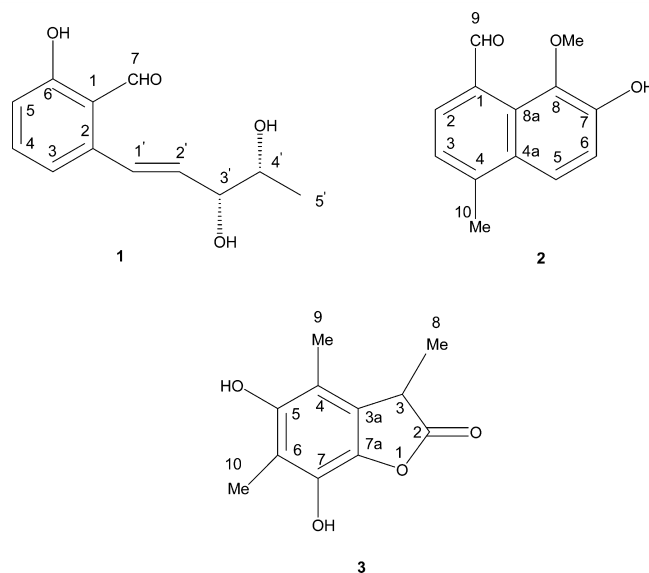

Figure 1. Structures of agropyrenol, agropyrenal, and agropyrenone (**1**, **2**, and **3**, respectively).

3*H*-benzofuranone, named agropyrenal and agropyrenone (**2** and **3**; Figure 1), respectively.[8]

Several studies were carried out to correlate the structure of microbial phytotoxins to their biological properties and to identify the active sites of the compounds. In particular, those carried out on phytotoxins with potential herbicidal activity were aimed at not only identifying the structural features important for the phytotoxicity but also producing derivatives with improved biological activities and biotechnological properties. Structure–activity relationship (SAR) studies were carried out on nonenolides (putaminoxins and stagonolides), cytochalasins, oxazatricyclakalenones, and alternethanoxins produced by fungi proposed for the control of *Erigeron annuus*,[9] *Bromus* sp., *Cirsium arvense*,[10,11] and *Sonchus arvensis*.[12]

Considering the originality of the agropyrenol chemical structure, containing a 6-substituted salycilic aldehyde ring and a 3,4-dihydroxy-1-pentenyl residue, and its phytotoxic properties associated with the lack of antimicrobial or zootoxic activities, it seemed of interest to further study this metabolite, trying to ascertain its potential as a novel natural herbicide and identify the structural features essential for its biological activity. For this reason, six derivatives (4–9; Scheme 1) were prepared by chemical transformation of the functionalities present in agropyrenol (1) and tested for their phytotoxic, antimicrobial, and zootoxic activities.

Scheme 1[a]
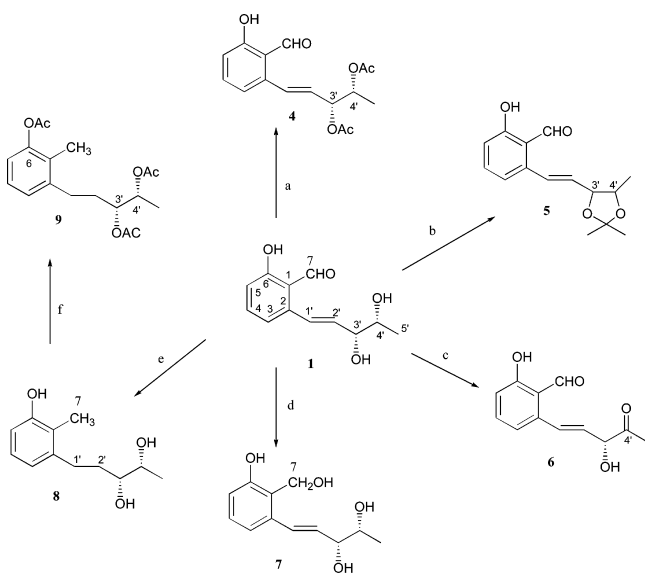
[a]Reagents and conditions: (a) Ac$_2$O, pyridine, room temperature (rt), 12 h; (b) dry Me$_2$CO, dry CuSO$_4$, rt, 18 h; (c) MnO$_2$, CH$_2$Cl$_2$, 25 °C, 1.5 h; (d) NaBH$_4$, MeOH, rt, 2 h; (e) H$_2$, Pd/C, MeOH, rt, 1 h; and (f) Ac$_2$O, pyridine, rt, 1.5 h.

MATERIALS AND METHODS

General Experimental Procedures. Infrared (IR) spectra were recorded as deposit glass film on a Perkin-Elmer (Norwalk, CT) spectrometer, and ultraviolet (UV) spectra were measured in MeCN, unless otherwise noted, on a Perkin-Elmer spectrophotometer. [1]H nuclear magnetic resonance (NMR) spectra were recorded at 600 or 400 MHz in CDCl$_3$ on Bruker (Kalsruhe, Germany) spectrometers. The same solvent was used as an internal standard. Electrospray ionization (ESI) mass spectra (MS) were recorded on an Agilent (Milano, Italy) Technologies 6120 Quadrupole LC/MS instrument. Analytical and preparative thin-layer chromatographies (TLCs) were performed on silica-gel (Kieselgel 60, F$_{254}$, 0.25 and 0.5 mm, respectively, Merck, Darmstadt, Germany) plates. The spots were

visualized by exposure to UV radiation (253) or by spraying first with 10% H$_2$SO$_4$ in MeOH and then with 5% phosphomolybdic acid in EtOH, followed by heating at 110 °C for 10 min. Column chromatography was performed on a silica-gel column (Merck, Kieselgel 60, 0.063–0.200 mm).

Production, Extraction, and Purification of Agropyrenol (1). To produce agropyrenol, the fungus *A. agropyrina* (Fairman) Trotter var. *nana* Punith, previously isolated from naturally infected leaves of *Elytrigia repens*, was used. The fungus, deposited in the culture collections of both the All-Russian Research Institute of Plant Protection, Pushkin, St. Petersburg, Russia (strain code A-10), and the Institute of Sciences of Food Production, Bari, Italy (strain code ITEM 12530), was grown on a mineral-defined liquid media named M1-D[13] as previously reported.[8] The purification of the organic extract obtained from its culture filtrate (8.6 L), carried out as previously described,[8] produced agropyrenol (1) as a yellow amorphous solid in sufficient amount (125.9 mg, 14.6 mg/L) for the preparation of the derivatives, as described in the successive sections of the present paper.

3′,4′-*O*,*O*′-Diacetyl Derivative (4). This compound was prepared by routine acetylation of compound 1 with acetic anhydride and pyridine as previously reported.[8]

3′,4′-*O*,*O*′-Isopropylideneagropyrenol (5). Compound 1 (10 mg) was dissolved in dry Me$_2$CO (10 mL) and kept in stirring conditions with dry CuSO$_4$ (400 mg) under reflux for 2 h. The mixture was then filtered, and the solvent was evaporated under reduced pressure to give an oily residue, which was purified by preparative TLC (silica gel, eluent CHCl$_3$), allowing us to obtain derivative 5 as a homogeneous compound (11 mg, R_f = 0.66). IR ν_{max}: 3604, 2868, 1643, 1610, 1572, 1451, 1237 cm^{-1}. UV λ_{max} nm (log ε): 347 (3.30), 274 (3.60), 230 (3.97). ^{1}H NMR: see Table 1. ESI–MS (+) *m/z*: 285 [M + Na]$^+$. ESI–MS (−) *m/z*: 261 [M − H]$^-$.

4′,*O*-Didehydroagropyrenol (6). Compound 1 (10 mg) dissolved in dry CH$_2$Cl$_2$ (3 mL) was oxidized with dry MnO$_2$ (82 mg) under stirring at room temperature for 1.5 h. The mixture was then filtered, and the solution was evaporated under reduced pressure to give an oily residue, which was purified by preparative TLC [silica gel, eluent CHCl$_3$/iPrOH (97:3, v/v)], giving derivative 6 as a homogeneous compound (3 mg, R_f = 0.69). IR ν_{max}: 3434, 2851, 1736, 1684, 1646, 1580, 1465 cm^{-1}. UV λ_{max} nm (log ε): 355 (3.05), 285 (3.40), 246 (3.62). ^{1}H NMR: see Table 1. ESI–MS (+) *m/z*: 177 [M − CH$_3$CO]$^+$. ESI–MS (−) *m/z*: 176 [M − CH$_3$CO − H]$^-$.

7,*O*-Dihydroagropyrenol (7). Compound 1 (5 mg) was dissolved in MeOH (200 µL) and reduced at room temperature with NaBH$_4$ (7 mg) for 2 h. The mixture was then diluted with water (10 mL), neutralized to pH 7 with 0.1 N HCl, extracted with EtOAc (4 × 15 mL), dehydrated with Na$_2$SO$_4$, and finally evaporated under reduced pressure, giving an oily residue. It was purified by preparative TLC [silica gel, eluent CHCl$_3$/iPrOH (1:1, v/v)] allowing us to obtain derivative 7 as a homogeneous compound (5 mg, R_f = 0.74). IR ν_{max}: 3395, 1659, 1577, 1462 cm^{-1}. UV λ_{max} nm (log ε): 253 (2.84), 216 (sh). ^{1}H NMR: see Table 1. ESI–MS (+) *m/z*: 263 [M + K]$^+$, 247 [M + Na]$^+$.

7,7,1′2′-Tetrahydro-7-deoxyagropyrenol (8). Compound 1 (5 mg) was first dissolved in MeOH (500 µL), then added to a presaturated 10% Pd/C (3 mg) suspension in the same solvent (500 µL), and hydrogenated at room temperature and atmospheric pressure under stirring conditions. The reaction was stopped after 1 h by filtration and evaporated under reduced pressure; the residue (2.8 mg) was purified by preparative TLC [silica gel, eluent CHCl$_3$/iPrOH (9:1, v/v)], giving derivative 8 as a homogeneous compound (2.5 mg, R_f = 0.53). IR ν_{max}: 3373, 1585, 1541, 1465 cm^{-1}. UV λ_{max} nm (log ε): 280 (1.81). ^{1}H NMR: see Table 1. ESI–MS (+) *m/z*: 249 [M + K]$^+$, 233 [M + Na]$^+$. ESI–MS (−) *m/z*: 209 [M − H]$^-$.

6,3′,4′-*O*,*O*′,*O*″-Triacetyl Derivative (9). Derivative 8 (8 mg) was acetylated with pyridine (50 µL) and Ac$_2$O (50 µL) at room temperature for 1.5 h. The reaction was stopped by the addition of MeOH. The azeotrope obtained by the addition of C$_6$H$_6$ was evaporated by a N$_2$ stream. The oily residue (12 mg) was purified by preparative TLC [silica gel, eluent CHCl$_3$/iPrOH (95:5, v/v)] to give the 7,3′,4′-*O*,*O*′,*O*″-triacetyl derivative (9) of agropyrenol as a

Table 1. ¹H NMR Data of Agropyrenol and Its Derivatives (1 and 5−9)ᵃ

position	1 — δ_H (J in Hz)	5 — δ_H (J in Hz)	6 — δ_H (J in Hz)	7 — δ_H (J in Hz)	8 — δ_H (J in Hz)	9 — δ_H (J in Hz)
3	6.90 d (8.4)	6.97 d (8.5)	7.09 d (7.8)	6.83 d (7.9)	6.63 d (7.8)	6.88 d (7.8)
4	7.44 dd (8.4 and 7.6)	7.46 dd (8.5 and 7.6)	7.57 t (7.8)	7.16 t (7.9)	6.99 t (7.8)	7.12 t (7.8)
5	6.93 d (7.6)	6.95 d (7.6)	7.11 d (7.8)	6.95 d (7.9)	6.78 d (7.8)	7.02 d (7.8)
7	10.3 s		10.42 s	5.00 s	2.21 s	2.33 s
1′	7.23 d (15.6)	7.22 d (15.6)	8.07 d (15.7)	6.87 d (15.6)	2.86 ddd (14.8, 10.2, and 5.3) 2.70 ddd (14.8, 9.9, and 6.4)	2.63 (2H) m
2′	6.12 dd (15.6 and 6.1)	6.07 dd (15.6 and 6.6)	6.65 dd (15.7 and 7.5)	6.01 dd (15.6 and 6.6)	1.75 m 1.68 m	1.82 (2H) m
3′	4.10 dd (6.4 and 6.1)	4.14 dd (8.4 and 6.6)	4.21 m	4.06 dd (6.6 and 6.4)	3.93 m	5.06 (2H) m
4′	3.71 quint (6.4)	3.90 dq (8.4 and 6.0)		3.73 quint (6.4)	3.64 quint (7.2)	5.06 (2H) m
5′	1.27 d (6.4)	1.33 d (6.0)	1.25 s	1.16 d (6.4)	1.20 d (7.2)	1.21 d (6.1)
OH	11.90 s	11.85 s	11.92 s		11.91 s	
OH′			9.79 d (7.4)			
Me₂C		1.46 s 1.48 s				
MeCO						2.11 s
MeCO						2.09 s
MeCO						2.07 s

ᵃThe chemical shifts are in δ values (ppm) from TMS.

homogeneous compound (11.0 mg, R_f = 0.76). IR ν_{max}: 1761, 1734, 1580, 1207 cm⁻¹. UV λ_{max} nm (log ε): 259 (2.46). ¹H NMR: see Table 1. ESI−MS (+) m/z: 359 [M + Na]⁺.

Biological Assays. A number of assays on different organisms were performed to preliminarily characterize the biological properties of the derivatives compared to the "source" main metabolite and, thus, to allow for a SAR study. Each metabolite was first dissolved in a minimum amount of MeOH (10⁻¹ M or not higher than 1% in the final solution) and then diluted with distilled water to the desired concentration. The following bioassays were performed.

Leaf Disk Puncture Assay. Agropyrenol (**1**) and its six derivatives (**4−9**) were tested using a leaf puncture assay on five weed species, i.e., four dicots [namely, *Chenopodium album* L., *Cirsium arvense* (L.) Scop., *Mercurialis annua* L., and *Sonchus oleraceus* L.] and one monocot [*Setaria viridis* (L.) P. Beauv.]. Pure compounds were tested at 2 mg/mL by applying 20 μL of solution to detached leaves previously punctured with a needle. Five replications (droplets) on separate leaves were used for each metabolite and for each plant species tested. Leaves were kept in a moistened chamber under continuous fluorescent lights. Symptoms were estimated visually between 3 and 5 days after droplet application, using a visual scale from 0 (no symptoms) to 4 (necrosis wider than 1 cm). Control treatments were carried out by applying droplets not containing the metabolites.

Seed Germination. The possible capability of agropyrenol and its derivatives to inhibit seed germination was assayed on seeds of *Setaria viridis*. Briefly, seeds were first sterilized with sodium hypochlorite (1% × 15 min) and then washed several times with sterile distilled water. Around 100 seeds were placed in each Petri dish on paper dishes (Whatman No. 4) imbibed with the metabolite solution (1 mL, 10⁻³ M) and incubated at 25 °C in the dark. Three replications were prepared per each compound. The number of germinated seeds were counted 5 days after treatment and expressed as the germination percentage in comparison to the untreated control.

Rootlet Elongation. This assay aimed at evaluating the possible inhibition of the rootlet growth, and it was carried out using tomato seeds. Briefly, tomato seeds were pretreated with sodium hypochlorite (1% × 10 min.) and placed in Petri dishes on filter paper disks imbibed with sterile distilled water for 5 days. Thus, 10 pre-germinated and uniform length seedlings were transferred to smaller dishes each containing the metabolite solution (2 mL, 10⁻³ M). Dishes were transferred to a growth chamber under continuous light. Three replicates were prepared for each sample. After 4 days, the rootlet length was measured and compared to the proper untreated control.

Chlorophyll Degradation and Frond Growth. Agropyrenol and derivatives were also tested on the aquatic plant *Lemna minor* for the possible capability to degrade chlorophyll and inhibit frond growth. The bioassay was carried out according to the protocol already described in detail.[14] All of the compounds were tested at 10⁻³ M.

Antimicrobial Bioassay. The antimicrobial activity was tested against three microorganisms using an agar diffusion assay according to the protocol already described.[15] In particular, the antifungal activity was tested on *Geotrichum candidum* grown on potato dextrose agar (PDA), whereas the antibiotic activity was assayed against *Bacillus subtilis* (a Gram-positive bacterium) grown on tryptic glucose yeast agar (TGYA, Biolife, Bothell, WA) and *Escherichia coli* (a Gram-negative bacterium) grown on Luria-Bertani (LB) agar (Sigma, St. Louis, MO). Up to 0.5 μM of each metabolite was applied per diskette. Three replications were performed for each compound. The eventual presence of an inhibition halo of the microbial growth was visually assessed 1 day after the application.

Zootoxic Activity Assay. The zootoxic activity was evaluated on *Artemia salina* L. larvae (brine shrimp) using the protocol already described.[15] The metabolites were tested at 4×10^{-4} M, with four replications for each compound. At 48 h after the assay was performed, the number of dead larvae was counted and toxicity was then expressed as a percentage of dead larvae referred to the total.

Statistical Analysis. All of the bioassays were performed twice with at least three replicates. When appropriate, standard deviation (SD) was determined.

■ RESULTS AND DISCUSSION

To determine the relationships between the structure of agropyrenol and its biological properties, identify the active sites of the compound, and possibly, increase its toxicity, six derivatives of the main fungal toxin were prepared. As previously reported,[8] compound **1** was converted into the corresponding 3′,4′-*O,O*′-diacetyl derivative (**4**; Scheme 1) by routine acetylation carried out with acetic anhydride and pyridine, which showed the reversible modification of the diol system of the 3,4-dihydroxy-1-pentenyl residue. Its conversion into the parent compound at physiological pH frequently occurs in other natural metabolites, and it is known as "lethal metabolism".[16] A different modification of the same diol system was obtained by conversion of compound **1** into the

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corresponding 3′,4′-O,O′-isopropylidene derivative (**5**; Scheme 1). The derivative **5** was obtained by reaction of compound **1** with dry Me$_2$CO and dry CuSO$_4$. Further, different modifications of the 3,4-dihydroxy-1-pentenyl residue were obtained by oxidation of agropyrenol with MnO$_2$. Although this reaction yielded three oxidized derivatives, only the major one (**6**, Scheme 1), showing the oxidation of the secondary hydroxy group at C-4′, was purified. The other two oxidized derivatives were obtained as minor products of this reaction. The ESI−MS of their mixture proved that they are mono-oxidized at C-3′ and dioxidized at C-3′ and C-4′, respectively. Unfortunately, they were not purified, despite the attempts made using high-performance liquid chromatography (HPLC). Furthermore, opposite to what is currently reported,[17] the mono-oxidized derivative **6** was also obtained when compound **1** underwent a reaction with NaIO$_4$, with the aim to obtain an oxidative cleavage of the diol system. Anyway, further mechanistic studies would be needed to clarify the course of this latter reaction. As expected, the reduction by NaBH$_4$ converted compound **1** into the corresponding 7,O-dihydro derivative (**7**; Scheme 1), with the conversion of the aldehyde group into the corresponding primary hydroxy group. The catalytic hydrogenation carried out in MeOH with 10% Pd/C at atmospheric pressure and room temperature allowed us to obtain the 7,7,1′,2′-tetrahydro-7-deoxy derivative (**8**; Scheme 1), which has not only the saturation of the double bond of the 3,4-dihydroxy-1-pentenyl residue, as expected, but also the complete and unusual reduction of the aldehyde group to the corresponding methyl group. This latter compound was converted into the 6,3′,4′-O,O′,O″-triacetyl derivative (**9**) by routine acetylation with pyridine and acetic anhydride, already used to convert compound **1** into derivative **4**.

The structures of all of the derivatives **4**−**9** were determined by comparing their spectroscopic data, essentially ^{1}H NMR (see Table 1) and ESI−MS (see the Materials and Methods), to those of agropyrenol.

Assayed by leaf puncture on detached leaves, derivatives **1**, **4**, and **5** caused severe to medium necrosis to weedy dicot plants. Derivatives **6**−**9** caused only moderate to nil effects on the same plants, whereas none of the compounds tested had effects on *S. viridis*.

From the analyses of results, it is possible to observe that all of the nonreversible modifications of the diol system of the 3′,4′-dihydroxypentenyl side chain at C-2 present in derivative **6** as well as that of the aldehyde group at C-1 present in derivatives **8** and **9** induced strong reduction or total loss of phytotoxicity. It is interesting to note that derivative **7**, in which only the aldehyde group at C-1 was reduced, produced some phytotoxicity on three of the five plants tested, while the activity was completely lost when also the 1,2-double bond of the 3,4-dihydroxypentenyl side chain was reduced, as in derivatives **8** and **9**. The activity showed by the diacetyl derivate of agropyrenol was not surprising, as cited above; it was probably hydrolyzed into compound **1** at physiological pH according to the "lethal metabolism".[16] A similar mechanism of hydrolysis could also convert derivative **5** into compound **1** and, therefore, justify its phytotoxicity. In fact, acetonide is stable at basic pH but hydrolyzes in acid conditions.[17]

When tested on seeds, particularly derivatives **5** and **4** caused a strong reduction of germination (60 and 48% reduction compared to the control, respectively). Agropyrenol (**1**) and derivative **8** had a modest effect (around 20−30%), whereas the other derivatives had a very modest or nil effect (Table 2).

Table 2. Biological Assays of Agropyrenol (1) and Its Derivatives (4−9)

| | | | | compound | | | | | | |
organism	type of organism	bioassay	effect measured	1	4	5	6	7	8	9
Cirsium arvense	weedy dicot plant	leaf puncture	necrosis[a]	3	3	4	2	2	0	0
Chenopodium album	weedy dicot plant	leaf puncture	necrosis	4	4	4	0	2	0	0
Mercurialis annua	weedy dicot plant	leaf puncture	necrosis	4	2	4	0	0	0	0
Setaria viridis	weedy monocot plant	leaf puncture	necrosis	0	0	1	0	0	0	0
Sonchus oleraceus	weedy dicot plant	leaf puncture	necrosis	2	2	4	0	2	2	0
Setaria viridis	weedy monocot plant	seeds	germination[b,c]	40.8 (0.3)	28.8 (1.1)	22.1 (1.7)	50.5 (2.2)	54.9 (0.1)	43.5 (0.9)	54.2 (0.4)
Lycopersicon esculentum	crop plant	germinated seeds	root elongation[c,d]	23.8 (11.9)	15.3 (7.1)	5.2 (2.7)	49.0 (21.0)	38.5 (14.0)	37.3 (9.9)	33.1 (9.7)
Lemna minor	aquatic plant	frond immersion	chlorophyll content[c,e]	6.4 (0.4)	5.3 (0.4)	2.5 (0.4)	7.3 (1.0)	7.1 (0.8)	8.5 (0.4)	8.8 (0.3)
Lemna minor	aquatic plant	frond immersion	fresh weight[c,f]	23.8 (2.8)	23.5 (1.8)	14.4 (4.1)	24.4 (3.5)	21.4 (2.3)	23.0 (1.6)	22.8 (1.9)
Artemia salina	aquatic crustacean	larvae	mortality (%)[g]	0	0	88 (4)	0	0	23 (2)	0
Geotrichum candidum	fungus	agar diffusion	inhibition halo[h]	–	–	+	–	–	–	–
Bacillus subtilis	Gram-positive bacterium	agar diffusion	inhibition halo	–	–	+	–	–	–	–
Escherichia coli	Gram-negative bacterium	agar diffusion	inhibition halo	–	–	–	–	–	–	–

[a]Data are expressed using a visual empiric scale from 0 = no symptoms to 4 = necrosis larger than 1 cm. [b]Data are expressed as a percentage of seed germination (SD in parentheses). [c]Mean values of controls: seed germination (SD in parentheses), 55% (2%); tomato root length, 50.1 mm (15 mm); chlorophyll content, 7.58 mg/L (1.81 mg/L); and fresh weight, 24.6 mg/well (3.5 mg/well). [d]Data are expressed in mm (SD in parentheses). [e]Data are expressed in mg/L (SD in parentheses). [f]Data are expressed in mg/well (SD in parentheses). [g]Data are expressed as a percentage of dead larvae on the total. [h]Results are reported as the presence/absence (+/−) of a growth inhibition halo.

Almost the same effects were observable in both the root elongation assay and the chlorophyll assay, in which derivatives **5** and **4** again proved to be the most effective compounds, causing a severe depletion of rootlet growth (90 and 69% reduction compared to the control, respectively, as deducible from Table 2) and an effective reduction in the chlorophyll content of *L. minor* fronds (67 and 31% reduction in comparison to the control, respectively; Table 2).

These results suggest that derivatives **4** and **5**, which are less polar than compound **1**, could be absorbed and pass across the cell membranes of the tested plants, before converting into compound **1** as described above.

Derivative **5** was active also in most of the bioassays on organisms other than plants. Indeed, it caused 88% mortality of brine shrimp larvae in the zootoxicity bioassay and a slight inhibition of fungal and Gram-positive bacterium growth. In these latter assays, only derivative **8** caused a negligible mortality to *A. salina* larvae, whereas all of the other derivatives were inactive (Table 2).

Probably to justify the zootoxic and antimicrobial activities of derivative **5** compared to compound **1**, it could be possible to invoke a similar mechanism to that proposed for its behavior in germination, root elongation, and chlorophyll content bioassays.

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Notes
The authors declare no competing financial interest.

■ REFERENCES

(1) Dayan, F. E.; Cantrell, C. L.; Duke, S. O. Natural products in crop protection. *Bioorg. Med. Chem.* **2009**, *17*, 4022−4034.

(2) Hüter, O. F. Use of natural products in the crop protection industry. *Phytochem. Rev.* **2010**, *10*, 185−194.

(3) Evidente, A.; Abouzeid, M. A. Characterization of phytotoxins from phytopathogenic fungi and their potential use as herbicides in integrated crop management. In *Handbook of Sustainable Weed Management*; Singh, H. P., Batish, D. R., Kohli, R. K., Eds.; Harworth Press, Inc.: New York, 2006; pp 507−533.

(4) Rimando, A.; Duke, S. O. Natural products for pest management. In *Natural Products for Pest Management*; Rimando, A., Duke, S. O., Eds.; American Chemical Society (ACS): Washington, D.C., 2006; ACS Symposium Series, Vol. *927*, Chapter 1, pp 2−21.

(5) Berestetskiy, A. A review of fungal phytotoxins: From basic studies to practical use. *Appl. Biochem. Microbiol.* **2008**, *44*, 453−465.

(6) Hansske, F.; Sterner, O.; Satadler, M.; Anke, H.; Dorge, L.; Shan, R. Papyracillic acid, method for preparation and its use as synthon for bioactive substances. U.S. Patent 5,907,047, 1999.

(7) Evidente, A.; Berestetskiy, A.; Cimmino, A.; Tuzi, A.; Superchi, S.; Melck, D.; Andolfi, A. Papyracillic acid, a phytotoxic 1,6-dioxaspiro[4,4]nonene produced by *Ascochyta agropyrina* var. *nana*, a potential mycoherbicide for *Elytrigia repens* biocontrol. *J. Agric. Food Chem.* **2009**, *57*, 11168−11173.

(8) Andolfi, A.; Cimmino, A.; Vurro, M.; Berestetskyi, A.; Troise, C.; Zonno, M. C.; Motta, A.; Evidente, A. Agropyrenol and agropyrenal, phytotoxins from *Ascochyta agropyrina* var. *nana*, a fungal pathogen of *Elytrigia repens*. *Phytochemistry* **2012**, *79*, 102−108.

(9) Evidente, A.; Capasso, R.; Andolfi, A.; Vurro, M.; Zonno, M. C. Structure−activity relationships studies of putaminoxins and pinolidoxins phytotoxic nonenolides produced by phytopathogenic *Phoma* and *Ascochyta* species. *Nat. Toxins* **1998**, *6*, 183−188.

(10) Berestetskiy, A.; Dmitriev, A.; Mitina, G.; Lisker, I.; Andolfi, A.; Evidente, A. Nonenolides and cytochalasins with phytotoxic activity against *Cirsium arvense* and *Sonchus arvensis*: A structure−activity relationships study. *Phytochemistry* **2008**, *69*, 953−960 (and references cited therein).

(11) Evidente, A.; Cimmino, A.; Andolfi, A.; Vurro, M.; Zonno, M. C.; Cantrell, C. L.; Motta, A. Phyllostictines A−D, oxazatricycloalkenones produced by *Phyllosticta cirsii*, a potential mycoherbicide for *Cirsium arvense*. *Tetrahedron* **2008**, *64*, 1612−1619.

(12) Evidente, A.; Punzo, B.; Andolfi, A.; Berestetskiy, A.; Motta, A. Alternethanoxins A and B, polycyclic ethanones produced by *Alternaria sonchi*, potential mycoherbicides for *Sonchus arvensis* biocontrol. *J. Agric. Food Chem.* **2009**, *57*, 6656−660.

(13) Pinkerton, F.; Strobel, G. A. Serinol as an activator of toxin production in attenuated cultures of *Helminthosporium sacchari*. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4007−4011.

(14) Cimmino, A.; Andolfi, A.; Zonno, M. C.; Troise, C.; Santini, A.; Tuzi, A.; Vurro, M.; Ash, G.; Evidente, A. Phomentrioloxin: A phytotoxic pentasubstituted geranylcyclohexentriol produced by *Phomopsis* sp. a potential mycoherbicide for *Carthamus lanatus* biocontrol. *J. Nat. Prod.* **2012**, *75*, 1130−1137.

(15) Bottalico, A.; Capasso, R.; Evidente, A.; Randazzo, G.; Vurro, M. Cytochalasins structure−activity relationship. *Phytochemistry* **1990**, *29*, 93−96.

(16) Hassal, K. A. *Biochemistry and Uses of Pesticides*; Verlag Chemie: Weinheim, Germany, 1990; pp 58, 72, 304, 429, and 497.

(17) Allinger, N. L.; Cava, M. P.; De Jongh, D. C.; Johnson, C. R.; Lebel, N. A.; Stevens, C. L. *Organic Chemistry*, 2nd ed.; Worth Publishers, Inc.: New York, 1976.