

Further secondary metabolites produced by the fungus *Pyricularia grisea* isolated from buffelgrass (*Cenchrus ciliaris*)

Marco Masi¹  | Ernesto Santoro²  | Suzette Clement³ | Susan Meyer³ |
Patrizia Scafato²  | Stefano Superchi²  | Antonio Evidente¹ 

¹Dipartimento di Scienze Chimiche,
Università di Napoli Federico II,
Complesso Universitario Monte S. Angelo,
Naples, Italy

²Dipartimento di Scienze, Università della
Basilicata, Potenza, Italy

³Shrub Sciences Laboratory, U.S. Forest
Service Rocky Mountain Research Station,
Provo, Utah, USA

Correspondence

Marco Masi and Antonio Evidente,
Dipartimento di Scienze Chimiche,
Università di Napoli Federico II,
Complesso Universitario Monte S. Angelo,
Via Cintia 4, 80126 Naples, Italy.
Email: marco.masi@unina.it; antonio.
evidente@unina.it

Stefano Superchi, Dipartimento di
Scienze, Università della Basilicata, Via
dell' Ateneo Lucano 10, 85100 Potenza,
Italy.
Email: stefano.superchi@unibas.it

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Abstract

The fungal pathogen *Pyricularia grisea* has been studied to evaluate its production of phytotoxins for the biocontrol of the buffelgrass (*Cenchrus ciliaris* L.) weed. A first investigation allowed to isolate several new and known phytotoxic metabolites. However, the further investigation on the organic extract obtained from the fungus liquid culture showed the presence of other metabolites possibly contributing to its phytotoxicity. Thus, four known metabolites were isolated and identified by spectroscopic (nuclear magnetic resonance [NMR] and high-resolution electrospray ionization mass spectrometry [HRESIMS]) methods as dihydropyriculol (**1**), *epi*-dihydropyriculol (**2**), 3-methoxy-6,8-dihydroxy-3-methyl-3,4-dihydroisocoumarin (**3**), and (*R*)-mevalonolactone (**4**). The absolute configuration of **1–3** was determined for the first time by a computational analysis of their electronic circular dichroism (ECD) spectra. When the isolated compounds were bioassayed at a concentration of 5×10^{-3} M in a buffelgrass coleoptile and radicle elongation test no toxicity was detected. On the contrary, compounds **1** and **3** showed a significant stimulating effect of radical elongation. Furthermore, the difference in growth stimulation between **1** and its epimer **2** highlights the tight relationship between absolute configuration and biological activity of these fungal metabolites.

KEYWORDS

absolute configuration, biopesticides, electronic circular dichroism, growth stimulating effect, TDDFT computations

1 | INTRODUCTION

Buffelgrass (*Cenchrus ciliaris* L.), also known as *Pennisetum ciliare* L. or African foxtail grass, is a perennial grass native to tropical and subtropical arid regions of Africa and western Asia.¹ For many years, it has been

considered one of the most important pastoral species for its high nutritional value, resistance to heavy grazing, and fast growth. These characteristics promoted its introduction in many semiarid regions of the world including northwestern Mexico and southwestern United States.² Today, it is considered a highly invasive non-native weed in some parts of its introduced range and is officially listed as a noxious weed.³ In the Saguaro National Park,

Dedicated in honor and to the memory of Professor Koji Nakanishi.

the increased fire frequency caused by this weed is decimating the iconic saguaro cactus, and the Sonoran Desert ecosystem is becoming a grassland monoculture.^{4,5} Physical removal with hand tools and the use of broad-spectrum herbicides (e.g., glyphosate) are the two methods employed to manage the buffelgrass invasion.⁶ Bioherbicide formulations based on the phytotoxins produced by pathogenic fungi of *C. ciliaris* could be adopted as an innovative biocontrol strategy to avoid the use of synthetic pesticides.^{7–10} Thus, two buffelgrass foliar pathogens have been isolated from diseased plants, and their ability to produce phytotoxic metabolites in vitro has been evaluated. From the culture filtrates of *Cochliobolus australiensis*, nine phytotoxic compounds have been isolated including a new dihydropyranopyran-4,5-dione, named cochliotoxin, and two new tetrasubstituted 3-chromanonacrylic acids, named chloromonilinic acids C and D.^{11,12} These metabolites were chemically characterized by spectroscopic methods, and their absolute configuration (AC) was assigned through chiroptical methods.¹³ Furthermore, their biological activity was evaluated at different concentrations using a leaf puncture assay on the host plant and on nonhost indigenous plants. Among them, the most promising phytotoxin resulted to be radicinin, which demonstrated low toxicity to native plants, no effects on zebrafish (*Brachydanio rerio*) embryos, but high target-specific toxicity on buffelgrass.¹⁴ Successively, an HPLC method for its quantification in complex mixtures has been developed,¹⁵ and some key hemisynthetic derivatives were prepared starting from radicinin to carry out a structure–activity relationship study.¹⁶ Recently, an efficient synthetic strategy for the obtainment of this metabolite or some its active analogues was developed.¹⁷ From the culture filtrates of *Pyricularia grisea*, two monosubstituted hex-4-ene-2,3-diols, named pyriculins A and B, were isolated together with (10*S*,11*S*)-(–)-*epi*-pyriculol, *trans*-3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone and (4*S*)-(+)-isoscлерone. The relative and AC of these compounds was determined by a combination of spectroscopic (nuclear magnetic resonance [NMR] and electronic circular dichroism [ECD]) and computational tools.¹⁸ When tested by leaf puncture assay on host plant and in a buffelgrass coleoptile and radicle elongation assay, (10*S*,11*S*)-(–)-*epi*-pyriculol proved to be the most toxic compound.¹⁸ However, the toxicity showed by the organic extract appeared consistent with the presence of other secondary metabolites as also showed by its chromatographic investigations. Thus, further studies were carried out on the metabolites of the organic extract of *P. grisea* culture filtrates allowing the isolation of other four secondary metabolites. Therefore, this manuscript reports the purification and the chemical identification of

these compounds. Moreover, taking into account the tight relationship between AC and biological activity of metabolites originating from plants and fungi,^{19,20} this manuscript reports for the first time the assignment of their absolute stereochemistry by computational analysis of ECD spectra and their activity against buffelgrass.

2 | MATERIALS AND METHODS

2.1 | General

Optical rotations were measured in solution on a JASCO (Tokyo, Japan) P-1010 digital polarimeter; ultraviolet (UV) and ECD spectra were recorded at room temperature on a JASCO (Tokyo, Japan) J815 spectropolarimeter, by using 0.1-mm cells and concentration of 6.87×10^{-3} M for **1** and 8.15×10^{-3} M for **2** in methanol, whereas compound **3** was measured in 4.5×10^{-3} M acetonitrile solution. ¹H and ¹³C NMR spectra were recorded using 1% CD₃OD in CDCl₃ at 400 and 100 MHz, respectively, on a Bruker (Karlsruhe, Germany) spectrometer, and CDCl₃ was used as an internal standard. High-resolution electrospray ionization mass spectrometry (HRESIMS) and electrospray ionization (ESI) spectra were recorded on Agilent (Santa Clara, CA, USA) 6230 Accurate-Mass TOF LC/MS instrument. Analytical and preparative thin-layer chromatography (TLC) were performed on silica gel (Kieselgel 60, F₂₅₄, 0.25 and 0.5 mm, respectively; Merck, Darmstadt, Germany) or on reverse phase (KC18 F₂₅₄, 0.20 mm; Whatman, Maidstone, UK) plates; the spots were visualized by exposure to UV light and/or iodine vapors and/or by spraying first with 10% H₂SO₄ in MeOH and then with 5% phosphomolybdic acid in EtOH followed by heating at 110°C for 10 min. Column chromatography (CC) was carried out on silica gel (Merck, Kieselgel 60, 0.063–0.200 mm).

2.2 | Fungal strain

The strain of *P. grisea* (SNM22) used in this study was isolated from diseased buffelgrass leaves as previously described.¹⁸

2.3 | Production, extraction, and purification of compounds 1–4

P. grisea was grown on potato dextrose broth (PDB) culture and extracted as previously described.¹⁸ The organic extract (212.8 mg) was purified by CC eluted with CHCl₃/*i*-PrOH (9:1, v/v), yielding 11 groups of

homogeneous fractions. The residue of some of these fractions was further purified yielding pyriculins A and B, (1*S*,11*S*)-(-)-*epi*-pyriculol, *trans*-3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone, and (4*S*)-(+)-isoscлерone, as previously described.¹⁸ Further purification of the residues of the eighth (60.2 mg) and tenth (51.3 mg) fractions allowed us to isolate other four compounds. In particular, the residue of the eighth fraction was further purified by TLC eluted two times with CHCl₃/MeOH (9:1, v/v) yielding two amorphous solids identified as 3-methoxy-6,8-dihydroxy-3-methyl-3,4-dihydroisocoumarin (**3**, 2.3 mg, Figure 1) and (*R*)-mevalonolactone (**4**, 3.1 mg, Figure 1). The residue of the tenth fraction was further purified by TLC using CHCl₃/EtOAc/MeOH (6:3:1, v/v) and then by TLC on reverse phase eluted five times with MeOH/H₂O (4:6, v/v) yielding two oily homogenous compounds identified as dihydropyriculol and *epi*-dihydropyriculol (**1** and **2**, 3.6 and 4.2 mg, Figure 1), as reported below.

2.4 | Dihydropyriculol (1)

[α]_D²⁵ + 6.2 (c 0.34 in EtOH); ¹H and ¹³C NMR were very similar to literature data²¹; HRMS (ESI, *m/z*): [*M* + *H*]⁺ calcd for C₁₄H₁₉O₄, 251.2983; found, 251.2988.

2.5 | *epi*-Dihydropyriculol (2)

[α]_D²⁵ -17.8 (c 0.36 in EtOH) [lit. Kono et al²¹: [α]_D²⁵ -19 (c 0.41 in EtOH)]; ¹H and ¹³C NMR were very similar to literature data²¹; HRMS (ESI, *m/z*): [*M* + *H*]⁺ calcd for C₁₄H₁₉O₄, 251.2983; found, 251.2980.

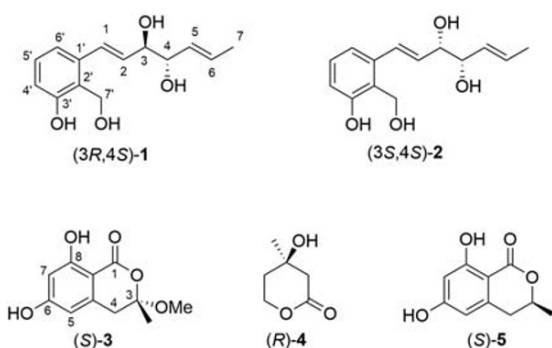


FIGURE 1 Structures and assigned stereochemistry of (+)-dihydropyriculol (**1**), (-)-*epi*-dihydropyriculol (**2**), 3-methoxy-6,8-dihydroxy-3-methyl-3,4-dihydroisocoumarin (**3**), (*R*)-mevalonolactone (**4**), and (+)-(*S*)-6-hydroxymellein (**5**)

2.6 | 3-Methoxy-6,8-dihydroxy-3-methyl-3,4-dihydroisocoumarin (3)

[α]_D²⁵ + 3.9 (c 0.30 in CHCl₃); ¹H and ¹³C NMR were very similar to literature data²²; HRMS (ESI, *m/z*): [*M* + *H*]⁺ calcd for C₁₁H₁₃O₅, 225.0763; found, 225.0773.

2.7 | (*R*)-mevalonolactone (4)

[α]_D²⁵ -17.5 (c 0.50 in CHCl₃) [lit. Varejão et al²³: [α]_D²⁵ -19.6 (c 0.60 in CHCl₃)]; ¹H and ¹³C NMR data were very similar to that previously described^{23,24}; HRMS (ESI, *m/z*): [*M* + *H*]⁺ calcd for C₆H₁₁O₃, 131.0708; found, 131.0713.

2.8 | Computational section

Preliminary conformational analyses were performed by the Spartan02 package²⁵ employing MMFF94s molecular mechanics (MM) force field with Monte Carlo searching and fixing arbitrarily ACs (3'*R*,4'*S*) for **1**, (3'*S*,4'*S*) for **2**, and (*S*) for **3**. All possible conformers were searched, considering the degrees of freedom of the system within an energy window of 30 kcal/mol. The minimum energy conformers found by MM were further fully optimized by Gaussian09 package²⁶ at the DFT/B3LYP/TZVP level, taking into account the solvent effect with IEFPCM implicit model.²⁷ The solvents used for calculations within the IEFPCM formalism were methanol for **1** and **2** and acetonitrile for **3**. All conformers are real minima, no imaginary vibrational frequencies have been found, and the free energy values have been calculated and used to get the Boltzmann population of conformers at 298.15 K. The DFT/B3LYP/TZVP/IEFPCM geometries were employed as input geometries for calculation of UV and ECD spectra at the TDDFT/CAM-B3LYP/aug-cc-pVDZ/IEFPCM level of theory. The computed UV and ECD spectra were obtained as average over the conformers Boltzmann populations. The ECD spectra were obtained by the Spec Dis package²⁸ from calculated excitation energies and rotational strengths, as a sum of Gaussian functions centered at the wavelength of each transition, with a parameter σ (width of the band at 1/2 height) of 0.3 eV. To guarantee origin independence and to evaluate the quality of the molecular wave functions employed, computed ECD spectra were obtained both in the length and velocity representation, using the lowest 30 excited states. The velocity/length calculated spectra were almost coincident, indicating a good level of calculation. Therefore, in all figures, only the velocity-form predicted spectra are reported. In order to facilitate the

correlation between experimental and calculated bands, which is sometimes hampered by misalignment of Cotton effects (CE), we have blue shifted the calculated spectra by 5 nm and scaled their intensity by dividing four times.

2.9 | Coleoptile and radicle elongation bioassay

Seeds of buffelgrass (*Cenchrus ciliaris*) were used for this assay, and compounds **1–4** were tested at a concentration of 5×10^{-3} M following the procedure previously described.¹⁸ In particular, the compounds were first dissolved in dimethyl sulfoxide (DMSO) and then brought to the desired concentration with distilled water (final DMSO concentration 2%). For the control treatment, the seeds were incubated in 2% DMSO. Three days after germination, the coleoptile and radicle length for each seedling were recorded using electronic calipers. Differential effects of the compounds on germination time and coleoptile and radicle length were evaluated using lsmeans separations from analysis of variance (SAS Proc GLM) on log-transformed data. Germination percentage was not replicated and was therefore not subjected to statistical analysis.

3 | RESULTS AND DISCUSSION

P. grisea was produced in liquid culture, and the culture filtrates were extracted as previously reported.¹⁸ The partial purification of the organic extract obtained allowed us to isolate pyriculins A and B, (10*S*,11*S*)-(–)-epipyriculol, *trans*-3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone, and (4*S*)-(+)-isosclerone, as previously described.¹⁸ Further purification of this organic extract, as detailed reported in Section 2, allowed us to isolate four other metabolites that were identified by comparing their physic and spectroscopic properties with those previously reported in literature. In particular, the ¹H and ¹³C NMR data of **1–4** (Figure 1) were very similar to those reported in literature for dihydropyriculol (**1**)²¹ and *epi*-dihydropyriculol (**2**),²¹ 3-methoxy-6,8-dihydroxy-3-methyl-3,4-dihydroisocoumarin (**3**),²² and (*R*)-mevalonolactone (**4**),^{23,24} respectively. Their identification was confirmed by the HRESIMS spectra and for **2** and **4** by optical rotation data. In fact, the HRESIMS spectrum of **1–4** showed the protonated forms $[M + H]^+$ at m/z 251.2988, 251.2980, 225.0773, and 131.0713, respectively. The optical rotation $[\alpha]^{25}_D -17.8$ (c 0.36 in EtOH) of **2** and that of **4** $[\alpha]^{25}_D -17.5$ (c 0.50 in CHCl₃) were very similar to those reported in literature for the natural *epi*-dihydropyriculol²¹ and (*R*)-mevalonolactone,²³

respectively. The AC of **1** was not reported before, whereas that of **2** was previously established by comparing its optical rotation with that of the product obtained by NaBH₄ reduction of *epi*-pyriculol,²¹ and finally, compound **3** was reported only in optically inactive form.²⁹

The AC assignment to **1–3** was therefore carried out. These are not crystalline compounds; therefore, the use of spectroscopic methods, allowing AC assignment in solution, was required. Moreover, we have recently demonstrated that the NMR-based Mosher approach is sometimes not reliable in the AC assignment to chiral diols,³⁰ whereas chiroptical methods, that is, ECD, vibrational circular dichroism (VCD), and optical rotatory dispersion (ORD), can provide more general and reliable tools for this task.³¹ At first, we attempted to apply for AC assignment to **1** and **2** the use of biphenyl chiroptical probes,^{32,33} that we recently demonstrated to be particularly straightforward for naturally occurring diols.³⁰ However, compounds **1** and **2** did not allow biphenyl derivatization, as a consequence of their low solubility in the required aprotic solvents. We then decided to employ for the AC assignment to those compounds the *ab initio* computational analysis of ECD spectra,³⁴ which proved to be a particularly reliable and straightforward approach for the AC assignment to complex and flexible natural products.^{35,36}

Accordingly, the UV and ECD spectra of the two compounds (+)-**1** and (–)-**2** were recorded in methanol in the 190–340 nm range (Figures 2 and 3). The UV spectra of both compounds are quite similar, displaying two main absorption bands at about 220 and 260 nm, followed by a much weaker broad band around 300 nm.

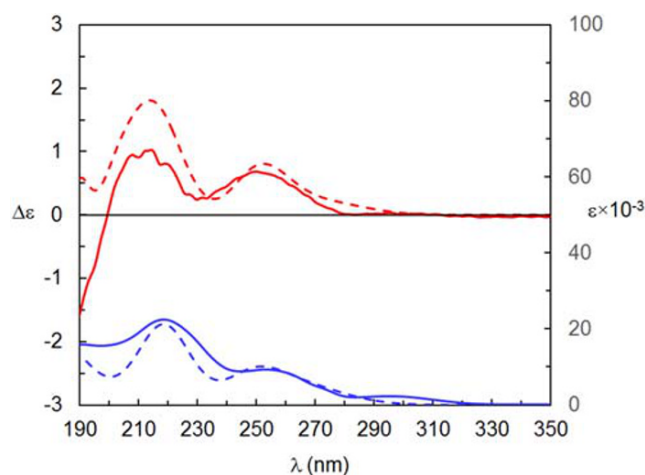


FIGURE 2 Experimental ultraviolet (UV) (solid blue line) and electronic circular dichroism (ECD) (solid red line) spectra of (+)-**1** recorded in methanol and theoretical UV (dashed blue line) and ECD (dashed red line) spectra for (3*R*,4*S*)-**1** (TDDFT/CAM-B3LYP/aug-cc-pVDZ/IEFPCM (MeOH))

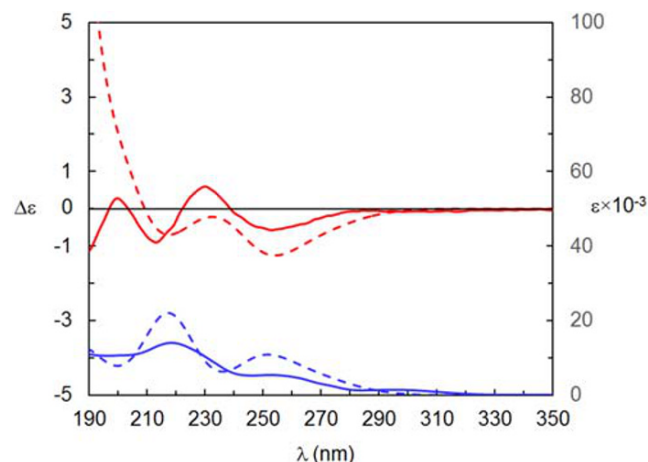


FIGURE 3 Experimental ultraviolet (UV) (solid blue line) and electronic circular dichroism (ECD) (solid red line) spectra of (–)-**2** recorded in methanol and theoretical UV (dashed blue line) and ECD (dashed red line) spectra for (3*S*,4*S*)-**2** (TDDFT/CAM-B3LYP/aug-cc-pVDZ/IEFPCM (MeOH))

The ECD spectrum of (+)-**1** displays two positive CE's at 250 ($\Delta\epsilon +0.7$) and 215 nm ($\Delta\epsilon +1.0$), approximately in correspondence of the two main UV bands, followed by a negative more intense band at 190 nm ($\Delta\epsilon -1.7$). The ECD spectrum of (–)-**2** shows instead several alternate in sign weak bands at 253 ($\Delta\epsilon -0.6$), 230 ($\Delta\epsilon +0.6$), 213 ($\Delta\epsilon -0.9$), and 200 nm ($\Delta\epsilon +0.3$). A computational conformational analysis of **1** and **2** was then carried out taking into account the (3*R**,4*S**)-**1** and (3*S**,4*S**)-**2** relative configurations established by NMR (*vide supra*). In this case, being the relative configuration known, it is possible to avoid to carry out computation on all diastereomers³⁷ considering only a single pair of enantiomers for the analysis.

The conformational analysis was first carried out at the MM level on the chosen theoretical models (3*R*,4*S*)-**1** and (3*S*,4*S*)-**2** (Figure 1), and the conformers found were further optimized by density functional theory (DFT) at DFT/B3LYP/TZVP/IEFPCM (MeOH) level of theory, taking into account the solvation effect by the integral equation formalism of the polarizable continuum model (IEPCM) approach.²⁷ Both compounds display relatively high flexibility, mainly on the chiral side-chain. In fact, computations provided 16 appreciably populated conformers for **1** (Table S1 and Figure S1) and 15 conformers for **2** (Table S2 and Figure S2). In both compounds, there is not a clearly prevalent conformer, but 10 ÷ 11 conformers between 20% and 3% population range. The UV and ECD spectra for (3*R*,4*S*)-**1** and (3*S*,4*S*)-**2** were calculated on previously found conformers at TDDFT/CAM-B3LYP/aug-cc-pVDZ/IEFPCM (MeOH) level of theory, again taking into account solvation in methanol by

IEFPCM and Boltzmann averaged over conformers populations. As inferred from Figure S3, the most populated conformers of (3*R*,4*S*)-**1** displays similar ECD spectra, but, although the majority have two positive CE's, three of them show opposite in sign negative CE's. Nevertheless, the Boltzmann averaged ECD spectrum nicely agrees in position and sign of the main CE's with the experimental one for (+)-**1** (Figure 2), clearly indicating the accuracy of the computed conformational analysis and the assignment of the (3*R*,4*S*) AC for this compound. The computed ECD spectra for the single conformers of (3*S*,4*S*)-**2** are even more different among them (Figure S4), but again, the main bands of Boltzmann averaged ECD spectrum are in a good agreement with those of the experimental one recorded for (–)-**2**, thus allowing to assign (3*S*,4*S*) AC to (–)-**2**. Dihydroisocumarin **3** was previously described only in optically inactive form,²⁹ and also, our sample showed an undetectable low $[\alpha]^{25}_D$ value. However, its ECD spectrum recorded in acetonitrile in the 190–350 nm range shows some clear, albeit quite weak, CE's (Figure 4). It is then probable that lactol **3** undergoes partial racemization under isolation condition, due to the lability of the acetal moiety under even weakly acidic conditions.

The UV spectrum of **3** appears very similar to those reported for 3-alkyl-6,8-dihydroxy isocumarins³⁸ like 6-hydroxymellein (**5**) (Figures 1 and S6) displaying a maximum at 217 nm due to the ¹L_a benzene transition, followed by a shoulder at 232 nm and by two more bands at longer wavelengths at 267 and 305 nm, allied to the lactone n- π^* and benzene ¹L_b transitions, respectively.^{39,40} The experimental ECD spectrum of **3** recorded

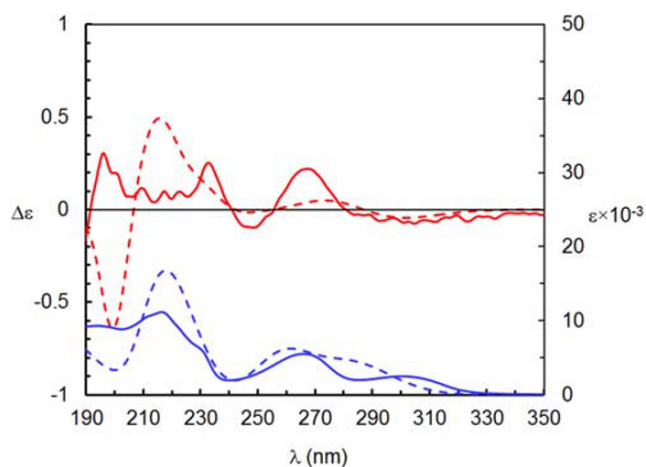


FIGURE 4 Experimental ultraviolet (UV) (solid blue line) and electronic circular dichroism (ECD) (solid red line) spectra of **3** recorded in acetonitrile and theoretical UV (dashed blue line) and ECD (dashed red line) spectra for (*S*)-**3** (TDDFT/CAM-B3LYP/aug-cc-pVDZ/IEFPCM (acetonitrile))

in the 190–350 nm range in acetonitrile (Figure 4) appears quite weak but clearly detectable in the 220–350 nm range. On the contrary, at higher energy is not possible detection of clear CE's as a consequence of the low dissymmetry factor g , resulting by the intense UV absorption band joined to the low ECD rotational strengths, in turn determined by the low enantiomeric purity. The detectable bands in the ECD spectrum are a positive CE at 232 nm ($\Delta\epsilon$ +0.5), a negative one at 248 nm ($\Delta\epsilon$ -0.1), a positive band at 268 nm ($\Delta\epsilon$ +0.2) and a broad weak band about 305 nm ($\Delta\epsilon$ -0.05). The ECD spectrum of **3** is almost superimposable to that of (*S*)-(+)-6-hydroxymellein (**5**)⁴¹ and in a mirror image relationship to that of its enantiomer (*R*)-(-)-**5** (Figure S7). This can tentatively allow to assign to our sample of **3** the same stereochemistry of (*S*)-(+)-**5**. For the configurational assignment of dihydroisocoumarins, a semiempirical rule has been reported, which correlates *P*-helicity of the lactone ring with a positive $n\text{-}\pi^*$ CE at around 260 nm.^{39–41} To apply this rule for the AC determination of **3**, a computational conformational analysis of this compound was carried out at DFT/B3LYP/TZVP/IEFPCM (acetonitrile) level of theory. Five appreciably populated conformers of (*S*)-**3** were found (Table S3 and Figure S5). In all the conformers, hydrogen bonding between the carbonyl moiety and the OH group in **6** position is present, and in the two most populated conformers, accounting for about 88% of the overall population, the C-3 methyl group is in equatorial position, whereas the OCH₃ is axial. In both conformers, the lactone ring then defines a *P*-helicity (Figure 5) which, according to the above rule, is allied to a positive CE at 260, in agreement with that experimentally found at 268 nm in the ECD spectrum of **3**. This, again support the assignment of (*S*) AC to the sample of **3**. Finally, to further support such assignment in a nonempirical way, a computational reproduction of the ECD spectrum was carried out. Therefore, the ECD spectra of the previously found conformers of (*S*)-**3** were computed at TDDFT/CAM-B3LYP/aug-cc-pVDZ/IEFPCM

(acetonitrile) level of theory and Boltzmann averaged over conformers populations. As inferred from Figure 4, the computed averaged ECD spectrum satisfactorily reproduce the experimental one in the 220–350 nm range thus definitively confirming the (*S*) AC for dihydroisocoumarin **3**.

When **1–4** were assayed on buffelgrass at a concentration of 5×10^{-3} M in a coleoptile and radicle elongation test, no toxicity was detected compared with the negative control (Figure 6). In particular, the coleoptile length of the seeds treated with **1**, **2**, and **4** did not significantly differ from the control, whereas those treated with **3** showed an increasing coleoptile development. Interestingly, the seeds treated with **1** and **3** had a significantly greater radical elongation development than the control, indicating a growth stimulating effect of these two compounds. These results, compared with those previously obtained testing *P. grisea* metabolites on buffelgrass,¹⁸ confirmed that until now, (10*S*,11*S*)-(-)-*epi*-pyriculol is the only metabolite produced by the fungus that showed phytotoxic activity against *C. ciliaris*. Thus, the presence of the aldehyde group at C-2 of (10*S*,11*S*)-(-)-*epi*-pyriculol still seems the most important structural feature to impart phytotoxicity.

Probably, the fungus produces compounds **1–4** and the other metabolites that showed no phytotoxicity, to compete with other organisms in its ecological niche. These results suggest to assay these metabolites to evaluate their antifungal and antibacterial activity against *P. grisea* competitors and other agrarian and forest pathogens to find their potential application. A further investigation is also needed to speculate on the growth-stimulating effect of **1** and **3**. In fact, another application may be their use as stimulants for breaking the dormancy of weed seeds in order to control the pests more successfully. This method is already used for the control of parasitic weeds (belonging to the genera *Striga*, *Pelipanche*, and *Orobancha* spp.) where suicidal germination agents (essentially strigolactones and orobanchols and synthetic analogues) are employed to stimulate their germination in the absence of a host causing the death of the seeds.

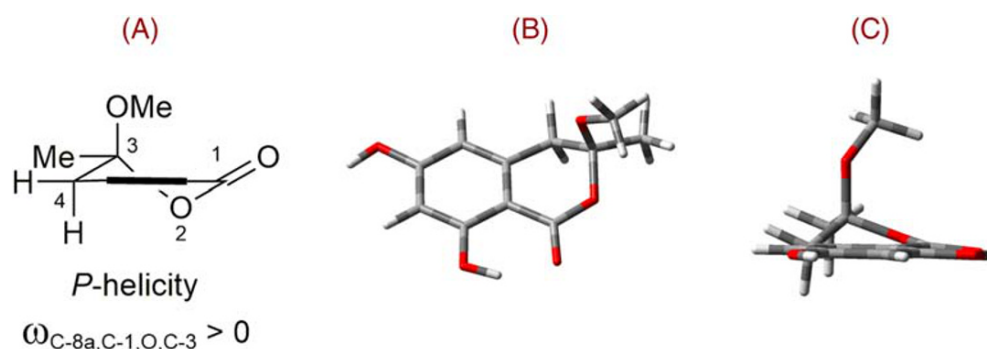


FIGURE 5 (A) *P*-helicity of (*S*)-**3** and density functional theory (DFT)-calculated most stable conformation **3a** (B) top view, (C) side view

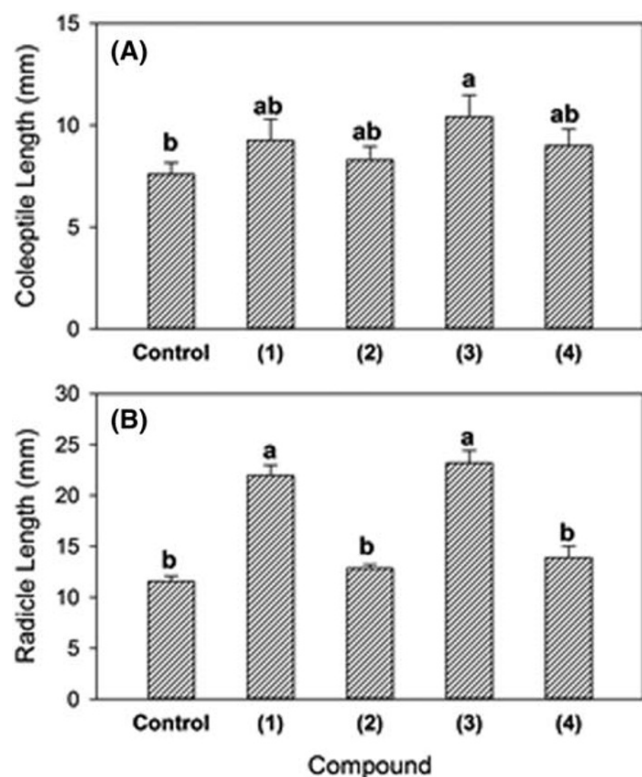


FIGURE 6 Phytotoxicity results of compounds 1–4 tested at a concentration of 5×10^{-3} M: (A) buffelgrass seedling shoot length elongation 3 days post-germination; (B) buffelgrass seedling root length elongation 3 days post-germination. Control: 2% dimethyl sulfoxide (DMSO) in distilled water. Within each panel, means headed by different letters are significantly different at $P < 0.05$ based on Lsmeans separations from mixed model analysis of variance

4 | CONCLUSION

Four other metabolites were isolated from the culture filtrates of *P. grisea* and identified as dihydropyriculol (1) and *epi*-dihydropyriculol (2), 3-methoxy-6,8-dihydroxy-3-methyl-3,4-dihydroisocoumarin (3), and (*R*)-mevalonolactone (4). For the first three only, the relative configuration was previously reported, and thus, their AC was assigned for the first time by a combining ECD chiroptical spectroscopy and computational techniques. Therefore, (3*R*,4*S*) AC was assigned to (+)-1, (3*S*,4*S*) to (–)-2 and (*S*) to 3. In particular, the latter compound was isolated herein for the first time in optically active form. Compounds 1 and 3 showed a growth-stimulating effect when were bioassayed in a buffelgrass radicle elongation test. These findings suggest that there may be additional applications for these compounds, but other studies are needed. Finally, the difference in growth stimulation showed by dihydropyriculol (1) and *epi*-dihydropyriculol (2) highlights the important relationship between AC

and biological activity already demonstrated for several natural products.^{19,20,42}

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ORCID

Marco Masi <https://orcid.org/0000-0003-0609-8902>

Ernesto Santoro <https://orcid.org/0000-0002-4276-4389>

Patrizia Scafato <https://orcid.org/0000-0002-6301-2856>

Stefano Superchi <https://orcid.org/0000-0002-7265-1625>

Antonio Evidente <https://orcid.org/0000-0001-9110-1656>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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