# Diplofuranones A and B, two further new 4-monosubstituted 2(3*H*)-dihydrofuranones produced by *Diplodia corticola*, a fungus pathogen of cork oak

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**Abstract**

Two new 4-monosubstituted 2(3*H*)-dihydrofuranones, named diplofuranones A and B, were isolated from liquid cultures of *Diplodia corticola*, a plant pathogenic fungus causing a canker disease of cork oak (*Quercus suber* L.). The same fungus also produces several metabolites such as the diplopyrone, the (3*S*,4*R*)-*trans*- and the (3*R*,4*R*)-*cis*-4-hydroxymellein, the sapinofuranone B and its (*S*,*S*)-enantiomer, the well known sphaeropsidins A-C, and the diplobifuranylones A and B. The diplofuranones A and B were characterised, using spectroscopic (essentially NMR and MS techniques) methods, as the 4-[(1*E*,3*E*)-5-hydroxyhexadienyl]butan-4-olide and its corresponding 3,4-dihydro side chain derivative. The stereochemistry of the stereogenic secondary hydroxylated carbon of the side chain of diplofuranone A was determined by application of Mosher's method and proved to be *R*. Diplofuranone A tested at 0.2 mg $mL^{-1}$ on non-host plant did not show phytotoxic activity.

**Keywords:** Cork oak, *Quercus suber* L., canker disease, *Diplodia corticola*, phytotoxic metabolites

## Introduction

*Diplodia corticola*, anamorph of *Botryosphaeria corticola* Phillips Alves et Luque, is an endophytic fungus widespread in Sardinian oak forests, and considered one of the main causes of cork oak (*Quercus suber* L.) decline.[1] The fungus can affect plants of different age, inducing

 

symptoms which include dieback, cankers and vascular necrosis. When inoculated on stems of young cork oak plants, *D. corticola* induced a slight collapse and dark brown discoloration of the cortical tissues around the inoculation site, a sudden wilting of the plant above it and subsequently a sprouting of secondary shoots below it.[2] These symptoms suggested that the fungus produced phytotoxic metabolites, as also observed for isolates of *D. mutila* from cypress and other oak species.[3] The main toxin, a new monosubstituted tetrahydropyranpyran-2-one, named diplopyrone, was isolated and chemically and biologically characterized from the culture filtrates of *D. corticola*.[4] Successively, the no empirical assignment of its absolute configuration has been approached by two different methods.[5] Recently, two 5'-monosubstituted tetrahydro-2*H*-bifuranyl-5-ones, named diplobifuranylones A and B, together with the (3*S,*4*R*)-*trans*- and the (3*R*,4*R*)-*cis*-4-hydroxymellein, the sapinofuranone B and its (*S*,*S*)-enantiomer and the well known sphaeropsidins A-C were reported as metabolites from the same fungus.[6] It is important underlined that sapinofuranone B and its (*S*,*S*)-enantiomer were obtained from the same fungal organic extract but in two independent experiments.

This paper describes the isolation and the chemical characterization of other two metabolites produced by *D. corticola*, which being structurally closed to sapinofuranones A and B (**5** and **6**, Figure 1), are named diplofuranones A and B (**1** and **2**, Figure 1).

## Results and Discussion

The organic extract obtained from culture filtrates of *D. corticola* was purified as described in the experimental section. From the most polar fraction of the second column were isolated the diplopyrone[4] and the diplobifuranylones A and B as recently described.[6] From the less polar fractions of the same column were isolated the sphaeropsidins A-C[3] and the sapinofuranone B already described together to sapinofuranone A[7] as phytotoxic metabolites produced by *Sphaeropsis sapinea* f.s. *cupressi* and *S. sapinea* phytopathogenic fungi on cypress (*Cupressus sempervirens* L.) and others conifers, respectively, and (*3S*,*4R*)-*trans*- and (*3R*,*4R*)-*cis*-hydroxymellein.[8-10]

The purification of the residue of fraction 5 of the initial column by TLC steps (see Experimental Section) yielded two metabolites as homogeneous oils resistant to crystallization, named diplofuranones A and B (**1** and **2**, 10 and 1.8 mg, **1** and 0.2 mg/l).

Diplofuranone A, assayed at 0.2 mg/l on non-host tomato plant, did not show phytotoxic activity. The phytotoxicity of diplofuranone B was not assayed due to the lacking of sufficient amount of this fungal metabolite. This inactivity did not surprise as **1**, in respect to the phytotoxic sapinofuranones A and B (**5** and **6**), showed a markedly modification of the 1-hydro-2,4-hexadienyl side chain at C-4, which thus showed its importance into impart the phytotoxicity.

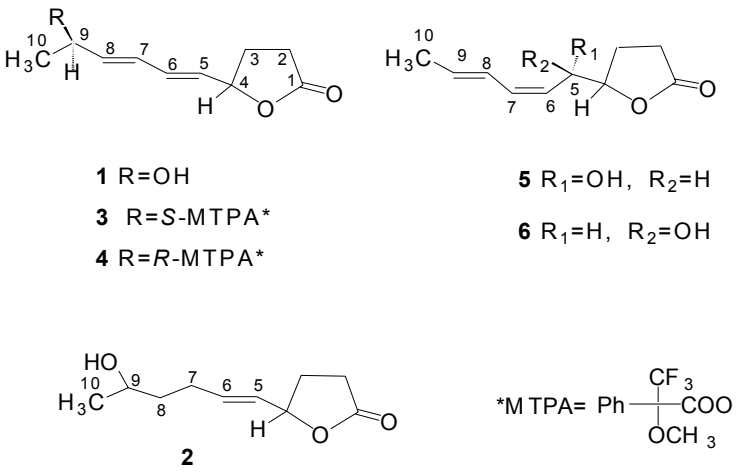


**Figure 1.** Structures of diplofuranones A and B (**1** and **2**), the MTPA esters of diplofuranone A (**3** and **4**), and sapinofuranones A and B (**5** and **6**).

Diplofuranone A (**1**, Figure 1) has a molecular formula of $C_{10}H_{14}O_3$, corresponding to four degrees of unsaturations, as deduced from the molecular weight of 182.0954, measured by HREIMS spectrometry. Absorption bands typical of γ-lactone carbonyl groups and hydroxy groups were observed in the IR spectrum,[11] while the UV spectra showed the absorption maximum of a dienyl residue at 230 nm.[12]

Preliminary $^1$H- and $^{13}$C-NMR spectra, compared to those of sapinofuranone A and B,[7] showed a similar pattern for the γ-lactone residue while those of the side chain at C-4 appeared strongly modified.

In particular, the $^1$H-NMR spectrum (Table 1) of diplofuranone A showed the multiplet of the protons of the two methylene groups ($CH_2$-2 and $CH_2$-3) and the quartet (*J*= 6.6 Hz) of the methyne (HC-4) group positioned, respectively, in α, β and γ in respect of the γ-lactone group, at the typical chemical shift values of δ 2.41 and 2.00, 2.55 and 2.00, and 4.98, respectively.[12] The latter proton (H-4) coupled in the COSY spectrum[13] with the protons of $CH_2$-3 and with the olefinic proton of the dienyl system present in the side chain attached at C-4. This latter (H-5) appeared as a double doublet (*J*= 14.7 and *J*= 6.6 Hz) at δ 5.65 as well as the other three dienyl protons (H-6, H-7 and H-8) resonating at δ 6.27 (*J*= 14.7 and *J*= 10.6 Hz), 6.25 (*J*= 14.9 and *J*= 10.6 Hz) and 5.83 (*J*= 14.9 and *J*= 6.1 Hz) and as expected coupling themselves in the COSY spectrum. In the same spectrum the olefinic proton at δ 5.83 also coupled with the proton of a secondary hydroxylated carbon (CH-9) appearing as a double quartet (*J*= 6.1 and *J*= 6.4 Hz) at δ 4.37 being also coupled with the terminal methyl group observed at δ 1.30 as a doublet (*J*= 6.4 Hz). In the $^{13}$C-NMR spectrum (Table 1) the carbons of the γ-lactone ring recorded at δ 176.8, 28.5, 28.7 and 80.3 (C-1, C-2, C-3 and C-4) were in perfect agreement with the values recorded for sapinofuranone A and B[7] and literature,[14] while the carbons of the side chain, in particular those of the dienyl system, were assigned on the basis of the couplings observed in HSQC spectrum.[14] Therefore, the doublets of olefinic carbons observed at δ 139.7, 132.2, 129.9 and

127.6 were attributed to C-8, C-6, C-5 and C-7, respectively, as well as the doublet and the quartet recorded at δ 68.2 and 23.2 were assigned to C-9 and C-10, respectively.[14]

On this basis diplofuranone A (**1**) can be formulated as the 4-[(1*E*,3*E*)-5-hydroxyhexadienyl]butan-4-olide. This structure was supported by the $^{1}H,^{13}C$ long-range correlations recorded for **1** in the HMBC spectrum (Table 1),[13] and by data of its MS spectra. The HREIMS spectrum, in addition to the molecular ion $[M]^{+}$at *m/z* 182.0954, showed the peaks generated from fragmentation typical of the γ-lactone and α-alkadienoyl substituted furan ring.[12,15] In fact, the molecular ion by successive loss of $H_2O$ and Me generated the ion at *m/z* 164 and 149, respectively.

**Table 1.** $^{1}H$- and $^{13}C$-NMR data of diplofuranones A and B (**1** and **2**). Chemical shifts reported as δ values from TMS[a]

| | **1** | | | **2** | | |
|---|---|---|---|---|---|---|
| Position | C[b] | H | HMBC | C[b] | H | HMBC |
| 1 | 176.8; s | | 2.55, 2.41 | 177.2; s | | 2.52, 2.39 |
| 2 | 28.5; t | 2.41; m<br>2.00; m | | 29.7; t | 2.39; m<br>1.98; m | 2.52, 1.98 |
| 3 | 28.7; t | 2.55; m<br>2.00; m | 5.65, 4.98, 2.41 | 28.0; t | 2.52; m<br>1.98; m | 5.53 |
| 4 | 80.3; d | 4.98; q (6.6) | 6.27, 5.65, 2.51, 2.41, 2.00 | 80.9; d | 4.91; q (6.8) | 5.83 |
| 5 | 129.9; d | 5.65; dd (14.7, 6.6) | 2.41, 2.00 | 130.7; d | 5.53; dd (15.1, 6.8) | 2.52, 1.98 |
| 6 | 132.2; d | 6.27; dd (14.7, 10.6) | 5.83, 4.98 | 128.0; d | 5.83; dt (15.1, 6.8) | 2.52 |
| 7 | 127.6; d | 6.25; dd (14.9, 10.6) | 5.65 | 27.9; t | 2.22; m (2H) | 5.53 |
| 8 | 139.7; d | 5.83; dd (14.9, 6.1) | 1.30 | 31.6; t | 2.22; m (2H) | 1.21 |
| 9 | 68.2; d | 4.37 ; dq (6.4, 6.1) | 6.25, 5.83, 1.30 | 67.8; d | 3.82; sex (6.3, 6.0) | |
| 10 | 23.2; q | 1.30; d (3H, 6.4) | 5.83 | 23.5; q | 1.21; d (3H, 6.0) | |

[a] 2D $^{1}H, ^{1}H$ (COSY) and 2D $^{13}C, ^{1}H$ (HSQC) NMR experiments delineated the correlations of all protons and the corresponding carbons

[b]Multiplicities determined by DEPT spectroscopy

Alternatively the molecular ion $[M]^+$, by loss of the alkadienoyl side chain generated the ion at *m/z* 85. In addition, the ions yielded by the side chain and that generated from it by $H_2O$ loss were recorded at *m/z* 97 and 79, respectively. The ESIMS(+) spectrum showed the potassium $[M+K]^+$ and the sodium $[M+Na]^+$ clusters at *m/z* 221 and 205, respectively.

Diplofuranone B (**2**, Figure 1) has a molecular formula of $C_{10}H_{16}O_3$ as deduced its HREIMS spectrum and spectroscopic properties (IR, NMR and MS spectra) similar to those described for **1**. Therefore, it differed in respect to **1** for the lack of unsaturation which was localized in the side chain. In fact, in the UV spectrum of **2** was absent the typical absorption maximum of a dienyl system observed in the same spectrum of **1**. Therefore, **2** showed a different side chain in respect to **1** as suggested also observing its $^1$H- and $^{13}$C-NMR spectra (Table 1). In fact, these latter differed from those of **1** only for the signal system of the side chain residue while those of the γ-lactone ring remained substantially unaltered. In particular, the $^1$H NMR spectrum showed the presence of only two coupled olefinic protons (H-6 and H-5) resonating at δ 5.83 and 5.53 as a double triplet (*J*= 15.1 and *J*= 6.8 Hz) and a double doublet (*J*= 15.1 and *J*= 6.8 Hz), respectively. The latter proton (H-5) coupled with the proton of the methyne group (HC-4) of the γ-lactone ring which as in **1** appeared as a quartet (*J*= 6.8 Hz) at a very similar chemical shift value of δ 4.91. The other olefinic proton (H-6) coupled with the protons of the adjacent methylene group ($H_2$C-7) resonating as a multiplet at δ 2.22 and these in turn with the protons of another methylene group ($CH_2$-8) always appearing as multiplet and at the same chemical shift value. These latter protons ($H_2$C-8), in turn, coupled with a sextet of a secondary hydroxylated carbon (HC-9), which, as expected, appeared upfield shifted (Δδ 0.55) in respect to **1** at δ 3.82, being also coupled with the terminal methyl group (Me-10) resonating as the doublet (*J*= 6.0 Hz) at δ 1.21.

The $^{13}$C-NMR spectrum (Table 1) showed signals very similar to those of **1** for the carbons of the γ-lactone ring but differed for those of the side chain. The olefinic carbons resonated at δ 130.7 and 128.0 (C-5 and C.6), while the carbons of the two new methylene groups, the secondary hydroxylated carbon and the terminal methyl group were recorded at expected chemical shifts values of δ 27.9, 31.6, 67.8 and 23.5 (C-7, C-8, C-9 and C-10).[14]

On the basis of these results diplofuranone B differed from **1** for the side chain and in particular for the lacking of the double bond between C-7 and C-8. Therefore, it can be formulate as 4-[(1*E*)-5-hydroxy-1-hexenyl]butan-4-olide (**2**, Figure **1**).

This structure was supported by the $^1$H,$^{13}$C long-range correlations recorded for **2** in the HMBC spectrum (Table 1),[13] and by data of its ESIMS spectrum, which showed the potassium $[M+K]^+$ and the sodium $[M+Na]^+$ cluster ions at *m/z* 223 and 207, respectively.

The stereochemistry of the double bonds of the side chain at C-4 of both **1** and **2** was deduced from the $^3J_{H,H}$ coupling constants that are consistent for all with a *trans*-stereochemistry.[12] The stereochemistry of the secondary hydroxylated carbon at C-9 of diplofuranone A was determined applying the Mosher's method.[16,17] Diplofuranone A by reaction with the *R*-(-)-α-methoxy-α-trifluorophenylacetate (MTPA) and *S*-(+)MTPA chlorides, was converted in the corresponding diastereomeric *S*-MTPA and *R*-MTPA esters (**3** and **4**,

Figure 1), whose spectroscopic data were consistent with the structure assigned to **1**. The comparison between the $^1$H-NMR data (Table 2) of the *S*-MTPA ester (**3**) and those of the *R*-MTPA ester (**4**) of **1** [δ*S*- δ*R*: (H-2')= -0.01; (H-3')= -0.01; (H-4)= -0.04; (H-5)= -0.02; (H-6)= -0.01; (H-8)= -0.01; (Me-10)= +0.03] allowed to assign, in agreement to the Mosher's method[17] and its further improvement,[18] a *R* configuration at C-9 of the side chain of **1**. Diplofuranone A can be formulated as 4-[(5*R*,1*E*,3*E*)-5-hydroxyhexadienyl]-3,4-dihydro-2H-furanone (**1**, Figure 1).

**Table 2.** $^1$H-NMR data of the (*S*)- and *(R)*-α-methoxy-α-trifluorophenylacetate (MTPA) esters of diplofuranone A and B (**3** and **4**, respectively). Chemical shifts reported as δ from TMS

| | **3** | **4** |
|---|---|---|
| **Position** | H | H |
| 2 | 2.42; m | 2.42; m |
| | 2.00; m | 2.01; m |
| 3 | 2.55; m | 2.55; m |
| | 2.00; m | 2.01; m |
| 4 | 5.75; m | 5.79; m |
| 5 | 5.63; dd (14.7, 6.4) | 5.65; dd (15.1, 7.3) |
| 6 | 6.23; dd (14.7, 10.3) | 6.24; d (15.1, 10.3) |
| 7 | 6.19; dd (14.7, 10.3) | 6.19; dd (15.6, 10.3) |
| 8 | 5.67; dd (14.2, 6.8) | 5.68; dd (15.6, 6.8) |
| 9 | 4.99; dq (7.3, 6.8) | 4.98; dq (6.8, 6.8) |
| Me | 1.32; d (6.4) | 1.36; d (6.4) |
| $OCH_3$ | 3.57; s | 3.54; s |
| Ph | 7.60-7.26; m | 7.60-7.26; m |

The absolute configuration of the two diplofuranones A and B could be assigned by the determination of the configuration of the stereogenic center (C-4) of the lactone ring applying the strategy of the *J*-based configurational analysis used for the related sapinofuranone A[19] or method based on the exciton analysis of the circular dichroism spectrum and the *ab initio* calculation of the optical rotatory power used for diplopyrone.[5] So, the results reported in this work allowed to restrict the possible stereoisomers, at least for **1,** to two diastereomers.

Considering the structures of sapinofuranones it is also possible to hypothesize a biosynthetic pathway, which starting from this fungal metabolites, leads to diplofuranones A and B as reported in Figure 2. The first step could be the protonation of the hydroxyl group at C-5 of the side chain attached at C-4 of the γ-lactone ring, followed by the elimination of a $H_2O$ molecule and the consequent shift of the double bond between C(6)-C(7) to C(5)-C(6) and that between C(8)-C(9) to C(7)-C(8) with the stereoselective attach of a $H_2O$ molecule at C-9. Finally, the deprotonation of the intermediate protonated alcohol generate the diplofuranone A (**1**). The

successive reduction of the double bond between C(7)-C(8) yielded the diplofuranone B (**2**). This hypothesized biosynthetic mechanism is in full agreement with the stereostructural features of (**1**) and (**2**) and allow to rule out the possibility that these two metabolites could be formed by sapinofuranones as an artefact of the work-up of the fungal culture filtrates. This biosynthetic mechanism was supported by the stereochemistry of C-9 and C(5)-C(6) and C(7)-C(8) double bonds, which have *E*-configuration in both (**1**) and consequently also in (**2**), and by the absence of other possible stereoisomers in the fungal culture filtrates.

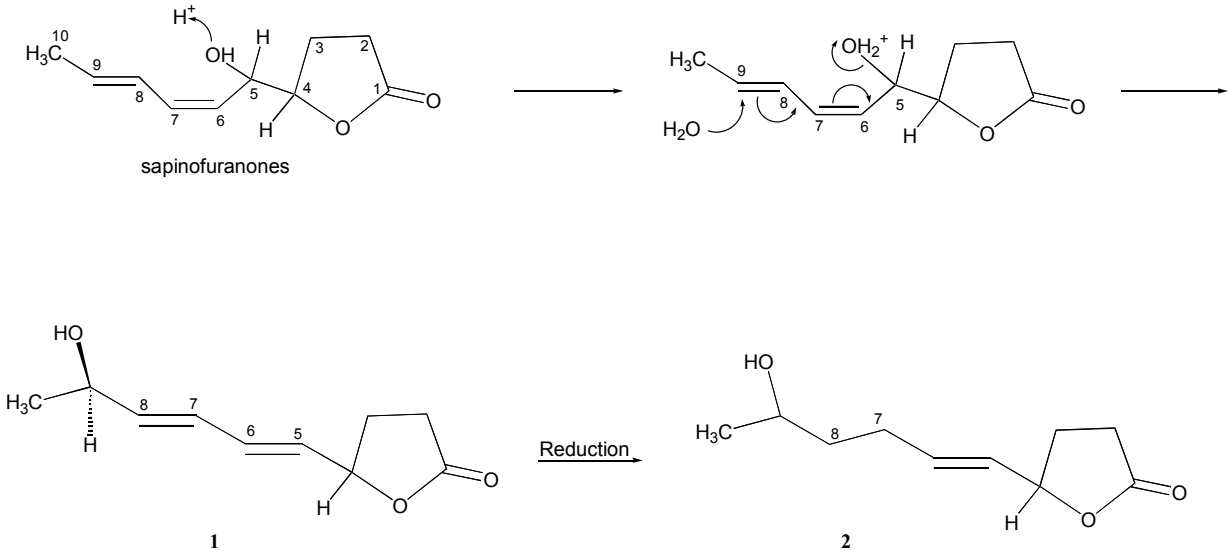


**Figure 2.** Biosynthetic pathway of sapinofuranones conversion into diplofuranones A and B (**1** and **2**)

Diplofuranones are strictly related to the sapinofuranones A and B isolated for the first time from *Sphaeropsis sapinea* infecting cypress tree[7] and the *S,S*-enantiomer of sapinofuranone B which was previously isolated from *Acremonium strictum,* a saprophytic fungus commonly found in soil and plant surfaces.[20] Butanolides are rare as naturally occurring compounds but are closely related to butenolides, which are well known as plant, fungal and lichen metabolites that also exhibit interesting biological activity.[21] Among these there are the seiridins, which are 3,4-dialkylbutenolides isolated from the culture filtrates of three species of *Seiridium* associated with the canker diseases of cypress. [22,23]

**Chemotaxonomic significance**

Independently from the phytotoxic activity, the occurrence of diplofuranones A (**1**) and B (**2**) may help to understand whether changes in the molecular structure of sapinofuranones affect its biological activity on host and non-host plants.[7] Furthermore, understanding of the secondary metabolism of *D. corticola* could help to elucidate the taxonomic relationship between *D. corticola* and *D. mutila*, the fungus most frequently isolated from branches and twigs of

declining oaks,[24] *S. sapinea* f. sp. *cupressi* [syn: *Diplodia pinea* (Desm) Kickx, Petrax et Sydow f. sp. *cupressi*] and *S. sapinea* (Fr.:Fr.) Dyko & Sutton an opportunistic pathogen of more than 30 species of *Pinus* in 25 countries.[25] In fact, it is important to point out that *D. corticola* produces diplopyrone, diplofuranones, diplobifuranylones, sphaeropsidins A-C, sapinofuranones and 4-hydroxymelleins while *D. mutila* produces sphaeropsidins A and C,[26] *S. sapinea* f. sp. *cupressi* produces sphaeropsidins A-F and sphaeropsidones[3], while *S. sapinea* only produces sapinofuranones A and B.[7] Therefore, *D. corticola* produces toxins in part similar to those (sphaeropsidins) produced by *D. mutila* and *S. sapinea* f. sp. *cupressi*, and those (sapinofuranones) of *S. sapinea*, but differ for the original biosynthesis of diplopyrone, the main phytotoxin, diplobifuranylones, diplofuranones, and the 4-hydroxymelleins.

## Experimental Section

**General Procedures.** Optical rotation was measured in $CHCl_3$ solution on a JASCO P-1010 digital polarimeter; IR and UV spectra were determined as neat and in $CH_3CN$ solution, respectively, on a Perkin-Elmer Spectrum ONE FT-IR spectrometer and a Perkin-Elmer Lambda 25 UV/Vis spectrophotometer; $^1$H- and $^{13}$C-NMR spectra were recorded at 400 MHz and 100 MHz, respectively, in $CDCl_3$, on Bruker spectrometers. The same solvent was used as internal standard. Carbon multiplicities were determined by DEPT spectrum.[13] DEPT, COSY-45, HSQC, HMBC experiments[13] were performed using Bruker microprograms. HREIMS and EIMS were taken 70 eV and on a Fisons Trio-2000 and a Fison ProSpec spectrometer, respectively. ESI MS were recorded on a Perkin-Elmer API 100 LC-MS; a probe voltage of 5300 V and a declustering potential of 50 V were used. Analytical and preparative TLC were performed on silica gel (Merck, Kieselgel 60, $F_{254}$, 0.25 and 0.5 mm respectively), or on reversed-phase (Merck, RP-18, $F_{254}$, 0.25 mm) plates. The spots were visualized by exposure to UV radiation and by spraying with 10% $H_2SO_4$ in MeOH and then with 5% phosphomolybdic acid in MeOH, followed by heating at 110 °C for 10 min. Column chromatography was performed on silica gel (Merck, Kieselgel 60, 0.063-0.20 mm).

**Fungal strain.** The *D. corticola* strain, used in this study, was isolated from stems of infected cork oak (*Q. suber*) trees collected in Sardinia (Italy). A single spore isolate of *D. corticola* was grown on potato-dextrose-agar slants at 25 °C for 10 days and then stored at 5 °C in the fungal collection of the "Dipartimento di Protezione delle Piante, Università di Sassari", Italy (PVS 114S).

**Production, extraction and purification of diplofuranones A and B.** The isolate PVS 114S of *D. corticola* was grown in stationary culture as previously reported[6]. The extraction of the culture filtrates (10 l) as well as the purification of the corresponding organic extract (960 mg) were carried out as previously reported[6]. This purification gave 11 groups of homogeneous fractions. The phytotoxic activity was concentrated in fractions 6-10. Successive purification of

the fraction 6 residue (90 mg), by silica gel column gave six combined fraction, of which only fractions 1, 4 and 5 showed phytotoxivc activity. The purification of the latter three fractions by combined column and TLC steps as previously detailed described,[6] gave the phytotoxin sphaeropsidins A,[27] B and C,[3] sapinofuranone B,[7] the (3*S*,4*R*)-*trans*- and (3*R*,4*R*)-*cis*-hydroxymellein,[9-11] the diplobifuranylones A and B[6] and the diplopyrone.[4] The residue of fraction 5 (59.6 mg) of the initial column purified by TLC steps (EtOAc-*n*-hexane, 1.5:1) yielded two metabolites as homogeneous oils resistant to crystallization, named diplofuranones A and B (**1** and **2**, 10 and 1.8 mg, 1 and 0.2 mg/l) [$R_f$ 0.37 and 0.36 and 0.46 and 0.14, eluent systems $CHCl_3$-*i*-PrOH (19:1), AcOEt-*n*-hexane (1.5:1), respectively].

**Diplofuranone A (1).** Colourless oil; $[\alpha]^{25}_D$ +9.0° (*c* 0.16); UV $\lambda_{max}$ nm (log ε): 230 (4.3); IR $\nu_{max}$ $cm^{-1}$:3422, 1770, 1650, 1182; $^1$H- and $^{13}$C-NMR: see Table 1; HREIMS (rel. int.) *m/z*: 182.0854 $[M]^+$ (6%) ($C_{10}H_{14}O_3$ requires 182.0843), 167 $[M\text{-}Me]^+$ (15), 164 $[M\text{-}H_2O]^+$ (28), 149 $[M\text{-}H_2O\text{-}Me]^+$ (84), 122 (100), 97 [$C_6H_9O$, side chain]$^+$ (81), 85 $[M\text{-}C_6H_9O]^+$ (71) 79 ($[C_6H_9O\text{-}H_2O]^+$ (90); ESIMS (+), *mz*: 221 $[M+K]^+$, 205 $[M+Na]^+$.

**Diplofuranone B (2)**. Colourless oil; $[\alpha]^{25}_D$ + 32.8° (*c* 0.11); UV $\lambda_{max}$nm (log ε): 225 (3.29); IR $\nu_{max}$ cm-1: 3477, 1768, 1698; $^1$H- and $^{13}$C-NMR: see Table 1; HREIMS (rel. int.) *m/z*: 184.1109 $[M]^+$ (9%) ($C_{10}H_{16}O_3$ requires 184.1099), 169 $[M\text{-}Me]^+$, (12), 166 $[M\text{-}H_2O]^+$, (24), 151 $[M\text{-}H_2O\text{-}Me]^+$, (75), 124 (100), 99 [$C_6H_{11}O$, side chain]$^+$, (79), 85 $[M\text{-}C_6H_{11}O]^+$ (67), 81 $[C_6H_9O\text{ -}H_2O]^+$ (88); ESIMS (+) (rel. int.) *m/z* 207 $[M+Na]^+$, 223 $[M+K]^+$.

**(*S*)-α-Methoxy-α-trifluorophenylacetate (MTPA) Ester of diplofuranone A (3).** (*R*)-(-)-MPTA-Cl (5 µl) was added to diplofuranone A (**1**, 1.5 mg), dissolved in dry pyridine (20 µl). The mixture was allowed to stand at room temperature. After 2 h, the reaction was complete, and MeOH was added. The pyridine was removed by a $N_2$ stream. The residue was purified by preparative TLC on silica gel (petroleum ether–$Me_2CO$, 2.3:1) yielding **3** as an oil (1.6 mg): $[\alpha]^{25}_D$ -30.3° (*c* 0.17); UV $\lambda_{max}$ nm log (ε): 230 (4.48); IR $\nu_{max}$ $cm^{-1}$: 1774, 1746, 1639, 1452, 1451, 1268, 1168; $^1$H-NMR: see Table 2;. ESIMS (+), *m/z*: 453 $[437]^+$, 421 $[M+Na]^+$, 399 $[MH]^+$.

**(*R*)-α-Methoxy-α-trifluorophenylacetate (MTPA) Ester of diplofuranone A (4).** (*S*)-(+)-MPTA-Cl (5 µl) was added to diplofuranone A (**1**, 1.5 mg), dissolved in dry pyridine (20 µl). The reaction was carried out under the same conditions used for preparing **3** from **1**. Purification of the crude residue by preparative TLC on silica gel (petroleum ether-$Me_2CO$, 2.3:1) yielded **4** as an oil (1.4 mg): $[\alpha]^{25}_D$ +40.3° (*c* 0.16); UV $\lambda_{max}$ nm (log ε): 231 (4.32) ; IR $\nu_{max}$ $cm^{-1}$ : 1774, 1746, 1452, 1269, 1169; $^1$H-NMR: see Table 2; ESIMS (+), *m/z*: 453 $[437]^+$, 421 $[M+Na]^+$, 399$[MH]^+$.

**Tomato cutting assay**. Diplofuranones A (**1**) was assayed for phytotoxicity on non-host plant (tomato: *Lycoperson esculentum* L. var. Marmande) as previously described.[6] The pure substance was dissolved in acetone and tested at concentrations of 0.05-0.2 mg m/l.

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