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Diplobifuranylones A and B, 5′-Monosubstituted Tetrahydro-2*H*-bifuranyl-5-ones Produced by *Diplodia corticola,* a Fungus Pathogen of Cork Oak

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Two new 5′-monosubstituted tetrahydro-2*H*-bifuranyl-5-ones, named diplobifuranylones A and B (**1** and **2**), were isolated from the culture filtrates of *Diplodia corticola*, the causal agent of a canker of cork oak (*Quercus suber*). The same fungus also produced eight known metabolites, namely, the diplopyrone, (3*S*,4*R*)-*trans*- and (3*R*,4*R*)-*cis*-4-hydroxymellein, sapinofuranone B and its (*S*,*S*)-enantiomer, and sphaeropsidins A−C. Diplobifuranylones A and B (**1** and **2**) were characterized, using spectroscopic and chemical methods, as two diastereomeric 5′-(1-hydroxyethyl)-3,4,2′,5′-tetrahydro-2*H*-[2,2′]bifuranyl-5-ones. While the relative stereochemistry of the two metabolites (**1** and **2**) was deduced by NOESY and ROESY experiments, the absolute stereochemistry of the chiral carbon of the hydroxyethyl side chain at C-5′, determined by application of Mosher's method, proved to be *S* and *R* in **1** and **2**, respectively. Assayed on a nonhost plant, diplobifuranylones A and B did not show phytotoxic activity. In an *Artemia salina* larvae lethality bioassay neither **1** nor **2** was toxic at the highest concentration tested (300 μg/mL).

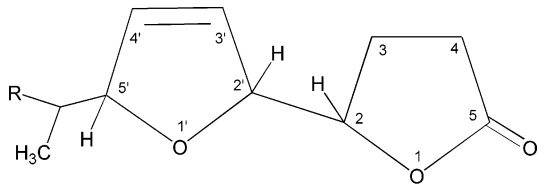
Diplodia corticola, anamorph of *Botryosphaeria corticola* Phillips Alves et Luque, is an endophytic fungus, widespread in Sardinian oak forests, and considered one of the main causes of cork oak (*Quercus suber* L.) decline.[1] The fungus can affect plants of different ages, inducing symptoms that include 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 and a sudden wilting of the plant above and subsequently a sprouting of secondary shoots below.[2] These symptoms suggest that the fungus produces phytotoxic metabolites, as also observed for isolates of *D. mutila* from cypress and other oak species.[3] Recently the main toxin, a new monosubstituted tetrahydropyranpyran-2-one, named diplopyrone, was isolated and chemically and biologically characterized.[4] Subsequently the nonempirical assignment of its absolute configuration has been approached by two different methods and assigned as 4a(*S*), 8a(*S*), 6(*R*), 9(*S*).[5]

This paper describes the isolation and the chemical and biological characterization of two other new metabolites (**1** and **2**) produced by *D. corticola*, together with eight known fungal toxins.

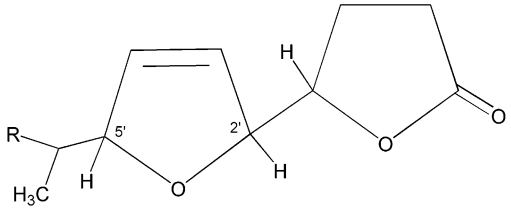
The organic extract obtained from culture filtrates of *D. corticola* was purified as described in the Experimental Section. From the less polar fractions of the second column were isolated sphaeropsidins A−C[3] and sapinofuranone B,[6] already described as phytotoxic metabolites produced by *Spheropsis sapinea* f.s. *cupressi* and *S. sapinea* phytopathogenic fungi on cypress (*Cupressus sempervirens* L.) and conifers, respectively. From the same fractions were also isolated the (*S*,*S*)-enantiomer of sapinofuranone B, which was isolated previously from *Acremonium strictum,* a saprophytic fungus commonly found in soil and plant surfaces,[7] and (3*S*,4*R*)-*trans*- and (3*R*,4*R*)-*cis*-4-hydroxymelleins.[8−10]

From the most polar fraction of the same column were further isolated diplopyrone[4] and two other new metabolites (0.37 and 0.42 mg/mL) obtained as homogeneous oils resistant to crystallization, which were named diplobifuranylones A and B (**1** and **2**) on the basis of the structural features shown below.

Diplobifuranylones A and B, assayed at 0.05−0.1 mg/mL on nonhost tomato plants, showed no sign of phytotoxicity. Compounds **1** and **2** were submitted to the brine shrimp (*Artemia salina* L.) lethality test in order to detect potential cytotoxicity to cancer cell lines. This simple and preliminary bioassay allows testing very small amounts of toxins, to compare the toxicity of different compounds, and also to quantify the effects. Both compounds were inactive against *A. salina* naupli at the highest concentration tested (300 μg/mL).



1 R=OH
3 R=*S*-MTPA
5 R=*R*-MTPA



2 R=OH
4 R=*S*-MTPA
6 R=*R*-MTPA

Diplobifuranylone A (**1**) was assigned a molecular formula of $C_{10}H_{14}O_4$, corresponding to four degrees of unsaturation, as deduced from the molecular weight of 221.0790, measured by HRESIMS for its adduct with sodium. Absorption bands typical of γ-lactone carbonyl groups and hydroxy groups were observed in the IR spectrum.[11] Preliminary NMR spectroscopic observation showed

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Table 1. ^{1}H and ^{13}C NMR Data of Diplobifuranylones A and B (**1** and **2**)[a,b]

	1			**2**		
position	δ_C, mult[c]	δ_H (J in Hz)	HMBC[d]	δ_C, mult[c]	δ_H (J in Hz)	HMBC[d]
2	80.1, CH	4.57, ddd (8.5, 5.4, 3.6)		80.1, CH	4.55, ddd (8.0, 5.3, 2.8)	
3	23.2, CH_2	2.25, m	2, 4, 5, 2′	23.7, CH_2	2.29, m	5, 2′
		2.20, m	2, 4, 5, 2′		2.22, m	2, 5, 2′
4	28.2, CH_2	2.59, ddd (16.7, 10.6, 7.0)	2, 3, 5	22.9, CH_2	2.66, ddd (16.7, 10.1, 7.0)	2, 3, 5
		2.44, ddd (16.7, 10.2, 6.5)	2, 3, 5		2.45, ddd (16.7, 10.3, 6.4)	2, 3, 5
5	177.4, qC			177.2, qC		
2′	75.6, CH	4.30, br s	3′	87.9, CH	4.97, m	4′, 5′
3′	132.4, CH	5.73, br d (10.3)	2′, 5′	128. 7, CH	5.92, br d (9.3)	2′, 5′
4′	126.1, CH	5.95, br d (10.3)	2′,	127.3, CH	6.01, br d (9.3)	2′, 5′
5′	69.1, CH	3.86, br d (7.5)	3′, 4′, Me*CH*, Me	90.9, CH	4.79 (m)	3′
Me*CH*	75.4, CH	3.38, dq (7.5, 7.3)	3′, Me	69.1, CH	3.90, dq (6.6, 3.4)	
Me	18.4, CH_3	1.32, d (7.3)	5′, Me*CH*	17.9, CH_3	1.18, d (6.6)	5′, Me*CH*
OH		5.10, br s		1.85, br s		

[a] The chemical shifts are in δ values (ppm) from TMS. [b] 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. [c] Multiplicities determined by DEPT spectrum. [d] HMBC correlations, optimized for 6 Hz, are from protons stated to the indicated carbon.

three out of four unsaturations in **1** were consistent with a γ-lactone carbonyl group and a *cis*-symmetrically disubstituted double bond. Analysis of the ^{1}H and ^{13}C NMR spectra (Table 1) confirmed these structural features. The ^{1}H NMR spectrum showed two coupled broad doublets (J = 10.3 Hz) at δ 5.95 and 5.73 due to H-4′ and H-3′, respectively, of a *cis*-disubstituted olefinic group, while the two protons of a methylene group (H_2C-4) positioned α with respect to the γ-lactone carbonyl group resonated as two doublets of double doublets (J = 16.7, 10.6, and 7.0 Hz and J = 16.7, 10.2, and 6.5 Hz, respectively) at the typical chemical shift values of δ 2.59 and 2.44.[12] The latter in the COSY spectrum[13] only coupled with themselves and with the multiplets of two protons of an adjacent methylene group (H_2C-3) at δ 2.25 and 2.20, respectively, which, in turn, proved to be coupled with the proton (H-2) appearing as a doublet of double doublets (J = 8.5, 5.4, and 3.6 Hz) at δ 5.57. The chemical shift values observed for this latter proton (H-2) as well as those of H_2C-3 were in good agreement with those reported for a methylene and oxygenated methine protons positioned β and γ with respect to the carbonyl group of a γ-lactone ring.[12] H-2 coupled in the same spectrum with a broad singlet (H-2′) resonating at δ 4.30, which in turn showed homoallylic coupling with a broad doublet (J = 7.5 Hz) due to the proton of another secondary oxygenated carbon (HC-5′) resonating at δ 3.86. H-2′ and H-5′ both coupled with the two olefinic protons (H-3′ and H-4′) and showed chemical shifts and coupling constant values typical of protons α-positioned in a 2,5-disubstituted 2,5-dihydrofuran ring.[12] Therefore, the fourth unsaturation was represented from this latter ring system. H-5′ also coupled with the secondary hydroxylated proton (MeC*H*) of the attached 1-hydroxyethyl side chain, appearing as a double quartet (J = 7.5 and 7.3 Hz) at δ 3.38, being also coupled with the terminal methyl group. This latter signal resonated as a doublet (J = 7.3 Hz) at δ 1.32, and the hydroxyl group signal appeared as a broad singlet at δ 5.10.[12]

The γ-lactone and the 2,5-dihydrofuran rings in **1** as well as the side chain attached to C-5′ were confirmed by correlations observed in the HSQC spectrum,[13] which allowed all signals of the ^{13}C NMR spectrum to be assigned (Table 1). This showed the presence of the carbonyl and the two methylene (CH_2-4 and CH_2-3) groups and the secondary oxygenated γ-carbon (HC-2) of the γ-lactone ring at typical chemical shift values of δ 177.4, 28.2, 23.2, and 80.1, respectively.[14] The same was verified for the carbons of the 2,5-dihydrofuran ring. In fact, the two olefinic and two secondary oxygenated carbons appeared at δ 132.4 and 126.1 (C-3′ and C-4′) and at δ 75.6 and 69.1 (C-2′ and C-5′), respectively.[14] Finally, the carbons of the 1-hydroxyethyl side chain resonated at δ 75.4 (Me*C*H) and 18.4 (*Me*CH), respectively.[14]

Thus diplobifuranylone A (**1**) is constituted by a 2-monosubstituted γ-lactone ring and a 2,5-disubstituted dihydrofuran ring joined through a C-2−C-2′ bond, with the side chain linked at C-5′ of the dihydrofuran ring. Therefore, **1** could be formulated as 5′-(1-hydroxyethyl)-3,4,2′,5′-tetrahydro-2*H*-[2,2′]bifuranyl-5-one.

This structure was supported by long-range correlations recorded for **1** in the HMBC spectrum (Table 1)[13] and by its MS data. The EIMS spectrum, in addition to the pseudomolecular ion $[M - H]^+$at *m*/*z* 197, showed fragmentation peaks typical of the γ-lactone and α-alkyl-substituted furan ring and probably generated the successive losses of CO_2 and CH_3CH_2O, followed by H_2O or CH_3O.[15]

Diplobifuranylone B (**2**) showed the same molecular formula of $C_{10}H_{14}O_4$, as deduced from its adduct with sodium in its ESIMS, and its spectroscopic properties (UV, IR, NMR, and MS spectra) were very similar to those described for **1**, but the optical rotation power was different. These preliminary data suggested that **2** could be a diastereomer of **1**. This was confirmed by analyzing the ^{1}H and ^{13}C NMR (Table 1), whose complete assignments were made from the COSY and HSQC NMR spectra. In particular, both the ^{1}H and ^{13}C NMR spectra differed from those of **1** only for the chemical shifts and multiplicity of the protons and carbons of C-2′ and C-5′, the carbons of the 2,5-dihydrofuran ring linked to the γ-lactone ring and to the hydroxyl ethyl side chain, which also showed significant changes in the chemical shifts of both carbons and protons. These results suggest a different stereochemistry essentially for the carbon of the 2,5-dihydrofuran ring bearing the two substituents, namely, the γ-lactone ring and the hydroxyl ethyl side chain, and a different stereochemistry of the chiral carbon (Me*C*H-OH) of the latter residue.

This was confirmed by comparing the NOESY spectra[13] of **1** and **2** reported in Table S1 (Supporting Information). The most significant effect was observed between H-2′ and H-5′ and between H-4′ and the terminal methyl of the side chain in diplobifuranylone B (**2**) and allowed the assignment of a relative *cis*-stereochemistry between C-2′ and C-5′ in **2**. The effect observed between H-2′ and H-5′ was also confirmed by a ROESY experiment[13] carried out on the same metabolite (**2**) but was not observed in diplobifuranylone A (**1**), which consequently should have a *trans*-stereochemistry between C-2′ and C-5′.

These results agreed with a Dreiding model inspection of both diplobifuranylones and were confirmed by the results of a series of double decoupling experiments carried out on **1** and **2**. The experiments allowed the recording of the coupling constants between H-2 and H-2′ (J = 3.6 and 2.8 Hz in **1** and **2**, respectively), especially the homoallylic coupling between H-2′ and H-5′, which proved to be J = 3.1 and 5.5 Hz in **1** and **2**, respectively, in agreement with the values reported in the literature for the *trans*- and the *cis*-coupling between H-2 and H-5 in a 2,5-dihydrofuran ring.[12,16]

The absolute stereochemistry of the secondary hydroxylated carbon of the 1-hydroxyethyl side chain at C-5′ was determined

applying the Mosher's method.[17−19] Diplobifuranylones A and B, by reaction with *R*-(−)-α-methoxy-α-trifluorophenylacetate (MTPA) and *S*-(+)MTPA chlorides, were converted in the corresponding diastereomeric *S*-MTPA and *R*-MTPA esters (**3** and **5** and **4** and **6**, respectively), whose spectroscopic data were consistent with the structure assigned to **1** and **2**. The comparison between the ^{1}H NMR data (Table S2, Supporting Information) of the *S*-MTPA ester (**3**) and those of the *R*-MTPA ester (**5**) of **1** [$\Delta\delta$ (**3**−**5**): H-5′ −0.01; H-4′ +0.10; H-3′ +0.05; H-2′ +0.01; *Me*CH +0.05] as well as those of the *S*-MTPA ester (**4**) and those of the *R*-MTPA ester (**6**) of **2** [$\Delta\delta$ (**4**−**6**): H-5′ −0.09; H-4′ −0.10; H-3′ −0.07; H-2′ −0.24; *Me*CH +0.05] allowed the assignment of an *S*- and *R*-configuration at Me*CH* of the side chain in **1** and **2**, respectively. Therefore, diplobifuranylones A and B (**1** and **2**) can be formulated as 5′-[(1*S*)-1-hydroxyethyl)-3,4,2′,5′-tetrahydro-2*H*-[2,2′]bifuranyl-5-one and its *R*-epimer at the chiral carbon of the side chain, respectively.

The lack of phytotoxicity observed testing diplobifuranylones A (**1**) and B (**2**) on nonhost tomato plants compared with the strong activity recorded for the structurally related sapinofuranones A and B on host (cypress and pine) and nonhost plants can be explained by the structural modification of the 1-hydroxy-2,4-hexadienyl side chain at C-4, which in **1** and **2** is converted into a *trans*- and *cis*-2,5-disubstituted 2,5-dihydrofuran ring, while the γ-lactone residues remain unaltered.

Bisfuranoids are a polyketide group of naturally occurring compounds that are broadly distributed in nature essentially as microbial metabolites produced by different fungal species such as *Aspergillus*, *Bipolaris*, *Cercospora*, *Chaetomium*, *Dothistroma*, *Farrowia*, and *Monocillium*. Most of these substances showed important biological activities like the aflatoxins.[20] This group of natural compounds presents two furan rings joined through one side, while bisfuranyls such as **1** and **2**, where the furan rings are joined through a bond, are only known as synthetic compounds.[21−24] Diplobifuranylones A (**1**) and B (**2**) represent the first example of monosubstituted natural bifuranyls joined through a bond.

Independently from the phytotoxic action, the occurrence of diplobyfuranylones A (**1**) and B (**2**) may help to understand whether changes in the molecular structure of sapinofuranones affect their biological activity on host and nonhost plants.[6] 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,[25] *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.[26] In fact, it is important to point out that *D. corticola* produces diplopyrone, diplobifuranylones, sphaeropsidins A−C, sapinofuranones, and 4-hydroxymelleins, *D. mutila* produces sphaeropsidins A and C,[3] *S. sapinea* f. sp. *cupressi* produces sphaeropsidins A−F and sphaeropsidones,[3] and *S. sapinea* produces only sapinofuranones A and B.[6] 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 in the original biosynthesis of diplopyrone, the main phytotoxin, diplobifuranilones, and the 4-hydroxymelleins.

Experimental Section

General Experimental Procedures. Optical rotation was measured in $CHCl_3$ solution on a JASCO P-1010 digital polarimeter. IR spectra were determined neat on a Perkin-Elmer Spectrum One FT-IR spectrometer. The UV spectra were recorded in CH_3CN solution on a Shimadzu UV-1601 UV−visible spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 600 or 400 MHz and at 150 or 100 MHz, respectively, on Bruker spectrometers with $CDCl_3$ used as internal standard. DEPT, COSY-45, HSQC, HMBC, NOESY, and ROESY experiments[15] were performed using Bruker microprograms. EIMS were taken at 70 eV on a Fisons Trio-2000 mass spectometer. HRESIMS were recorded on a Waters Micromass Q-Tof Micro instrument. ESIMS were recorded on a Perkin-Elmer API 100 LC-MS with a probe voltage of 5300 V and a declustering potential of 50 V. Analytical and preparative TLC were performed on silica gel (Merck, Kieselgel 60, F254, 0.25 and 0.5 mm, respectively) or on reversed-phase (Merck, RP-18, F254, 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 March 1996 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).

Extraction and Isolation. The isolate PVS 114S of *D. corticola* was grown in stationary culture 1 L Roux flasks containing 150 mL of Czapek medium with the addition of 0.5% yeast extract (pH 5.9). The flasks were incubated at 25 °C for 30 days in the dark. At harvest, the mycelium mat was removed by filtration. The culture filtrate (10 L; pH 7.5−8.1) was acidified to pH 4 with 2 N HCl and extracted with EtOAc (5 × 2 L). The combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give a red-brown oil residue with high phytotoxic activity. The crude residue (960 mg) was fractionated by column chromatography eluted with $CHCl_3$−*i*-PrOH (19:1). Fractions (8 mL each) were collected and pooled on the basis of their TLC profiles to yield 11 major fractions (1−12). Only the residues left from fractions 6−10 showed high phytotoxic activity on tomato cuttings. Phytotoxic fraction 6 (90 mg) was applied to a silica gel column that was eluted with petroleum ether−Me_2CO, 1:1. Six fractions were obtained.

The phytotoxic activity on tomato cuttings was concentrated in fractions 1, 4, and 5. Purification of fraction 1 (8.3 mg) by preparative TLC, eluting with $CHCl_3$−*i*-PrOH (19:1), gave the phytotoxin sphaeropsidin A[3] (6.0 mg). Purification of fraction 4 (32 mg) by preparative TLC, eluting with $CHCl_3$−*i*-PrOH (13:1), gave the phytotoxic sphaeropsidins B and C[3] (1.3 and 1.2 mg, respectively) and sapinofuranone B[6] (7.4 mg), its (*S*,*S*)-enantiomer[7] (4 mg), and another band (11 mg). The latter was fractionated by preparative TLC, using as eluent AcOEt−*n*-hexane (2.5:1), to obtain a mixture of two metabolites, which was separated by reversed-phase TLC using H_2O−MeCN (1.5:1) and identified as (3*S*,4*R*)-*trans*- and (3*R*,4*R*)-*cis*-4-hydroxymellein[8−10] (1.5 and 2.3 mg, respectively). Purification of fraction 5 (25 mg) by preparative TLC (eluent $CHCl_3$−*i*-PrOH, 93:7) yielded diplobifuranylones A and B (**1** and **2**, 3.7 and 4.2 mg, 0.37 and 0.42 mg/L, respectively), as two homogeneous oils [R_f 0.52 and 0.16 and 0.39 and 0.10, eluent systems $CHCl_3$−*i*-PrOH (93:7) and AcOEt−*n*-hexane (1.5:1) for **1** and **2**, respectively], and diplopyrone[4] (**7**, 11 mg).

Diplobifuranylone A (1): colorless oil; $[\alpha]^{25}_D$ −32.5 (*c* 0.19); UV λ_{max} (log ϵ) 196 (3.90) nm; IR ν_{max} 3417,1749, 1626 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 197 $[M - H]^+$ (9) 181 $[M - OH]^+$ (13), 167 $[M - CH_3O]^+$ (72), 154 $[M - CO_2]^+$ (94), 152 $[M - H - CH_3CH_2O]^+$ (95), 136 $[M - CO_2 - H_2O]^+$ (59), 134 $[M - H - CH_3CH_2O - H_2O]^+$ (81), 123 $[M - CO_2 - CH_3O]^+$ (88), 113 (100), 108 $[M - H - CO_2 - CH_3CH_2O]^+$ (95); ESIMS (+) *m*/*z* 237 $[M + K]^+$, 221 $[M + Na]^+$, 180 $[M - H_2O]^+$; ESIMS (−) *m*/*z* 197 $[M - H]^-$; HRESIMS (+) *m*/*z* 221.0790 $[M + Na]^+$ (calcd for $C_{10}H_{14}O_4Na$, 221.0790), 180 $[M - H_2O]^+$.

Diplobifuranylone B (2): colorless oil; $[\alpha]^{25}_D$ −90.7 (*c* 0.55); UV λ_{max} (log ϵ) 194 (3.83) nm; IR ν_{max} 3431, 1766, 1605 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 198 $[M]^+$ (19) 180 $[M - H_2O]^+$ (18), 167 $[M - CH_3O]^+$ (7), 152 $[M - H - CH_3CH_2O]^+$ (100), 136 $[M - CO_2 - H_2O]^+$ (47), 134 $[M - H - CH_3CH_2O - H_2O]^+$ (75), 123 $[M - CO_2 - CH_3O]^+$ (23), 111 $[M - CO_2 - CH_3CO]^+$ (98); ESIMS (+) *m*/*z* 237 $[M + K]^+$, 221 $[M + Na]^+$, 180 $[M - H_2O]^+$; HRESIMS (+) *m*/*z* 221.0784 $[M + Na]^+$ (calcd for $C_{10}H_{14}O_4Na$, 221.0790).

(*S*)-α-Methoxy-α-trifluorophenylacetate (MTPA) Ester of Diplobifuranylone A (3). (*R*)-(−)-MPTA-Cl (5 μL) was added to diplobifuranylone A (**1**, 1.5 mg), dissolved in dry pyridine (50 μL). The mixture was allowed to stand at room temperature. After 1 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, 1.3:1), yielding **3** as an oil (1.6 mg): $[\alpha]^{25}_D$ −47.6 (*c* 0.15); UV λ_{max} log (ϵ) 264 (2.67) nm; IR ν_{max} 1776, 1747, 1623, 1495, 1451, 1269, 1248 cm^{-1}; ^1H NMR, see Table S2 (Supporting Information); EIMS *m/z* 415 $[M + H]^+$ (0.2), 371 $[M + H - CO_2]^+$ (2.4), 355 $[M - CO_2 - Me]^+$ (4), 343 $[M + H - CO_2 - CO]^+$ (0.7), 282 $[M + H - CO - PhCO]^+$ (91), 189 $[PhC(OCH_3)CF_3]^+$ (100), 119 $[PhCOCH_3 - H]^+$ (61); ESIMS (+) *m/z* 453 $[M + K]^+$ 437 $[M + Na]^+$, 432 $[M + H_2O]^+$, 414 $[M]^+$.

(*S*)-α-Methoxy-α-trifluorophenylacetate (MTPA) Ester of Diplobifuranylone B (4). (*R*)-(−)-MPTA-Cl (5 μL) was added to diplobifuranylone B (**2**, 1.0 mg), dissolved in dry pyridine (50 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, 1.3:1) yielded **4** as an oil (1.4 mg): $[\alpha]^{25}_D$ −63.2 (*c* 0.14); UV λ_{max} log (ϵ) 266 (2.48) nm; IR ν_{max} 1775, 1747, 1623, 1493, 1451, 1270 cm^{-1}; ^1H NMR, see Table S2 (Supporting Information); EIMS *m/z* 415 $[M + H]^+$ (0.1), 369 $[M - H - CO_2]^+$ (0.3), 354 $[M - H - CO_2 - Me]^+$ (0.5), 282 $[M + H - CO - PhCO]^+$ (2.8), 188 $[PhC(OCH_3)CF_3 - H]^+$ (100), 119 $[PhCOCH_3 - H]^+$ (19); ESIMS (+) *m/z* 453 $[M + K]^+$, 437 $[M + Na]^+$, 432 $[M + H_2O]^+$, 415 $[M + H]^+$.

(*R*)-α-Methoxy-α-trifluorophenylacetate (MTPA) Ester of Diplobifuranylone A (5). (*S*)-(+)-MPTA-Cl (5 μL) was added to diplobifuranylone A (**1**, 1.5 mg) and dissolved in dry pyridine (50 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, 1.3:1) yielded **5** as an oil (1.7 mg): $[\alpha]^{25}_D$ −30.25 (*c* 0.12); UV, IR, and EIMS were very similar to those of **3**; ^1H NMR, see Table S2 (Supporting Information).

(*R*)-α-Methoxy-α-trifluorophenylacetate (MTPA) Ester of Diplobifuranylone B (6). (*S*)-(+)-MPTA-Cl (5 μL) was added to diplobifuranylone B (**2**, 1.0 mg) and dissolved in dry pyridine (50 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, 1.3:3) yielded **6** as an oil (1.2 mg): $[\alpha]^{25}_D$ +77.9 (*c* 0.33); UV, IR, and EIMS were very similar to those of **4**; ^1H NMR, see Table S2 (Supporting Information).

Brine Shrimps Lethality Bioassay. The brine shrimp (*Artemia salina*) bioassay was set up in 24-well culture plates (Iwaki, Japan) as reported by Epply[27] with some modifications. Compounds **1** and **2** were dissolved in methanol and tested in a concentration range of 30−300 μg/mL. Control wells with MeOH were included in the experiment. Tests were performed in quadruplicate. The percentage of larvae mortality was determined after exposure to the compounds for 24, 36, and 48 h at 26 °C.

Tomato Cutting Assay. Compounds **1** and **2** were assayed for phytotoxicity on nonhost plant (tomato: *Lycoperson esculentum* L. var. Marmande), as previously described.[4] The pure substances were dissolved in acetone and tested at concentrations of 0.05−0.1 mg/mL.

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Note Added after ASAP Publication: A compound was misnamed in the abstract in the version posted on March 1, 2006. The correct version appears on March 3, 2006.

Supporting Information Available: Tables of NOESY NMR spectral data for compounds **1** and **2** and ^1H NMR data of the MTPA esters **3**−**6**. This information is available free of charge via the Internet at http://pubs.acs.org.

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