Synthesis and Antifungal Activity of Aromatic Bis-γ-lactones Analogous to Avenaciolide

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Avenaciolide is a bis-γ-lactone isolated from *Aspergillus avenaceus* and possesses antifungal activity. Here, we describe the total syntheses and characterization by elemental analyses, and IR and NMR spectroscopy of three new bis-γ-lactones analogous to avenaciolide, where the octyl group of the natural product was replaced by aromatic groups. The effects of the avenaciolide, the novel compounds, and their synthetic precursors on the mycelia development and conidia germination of *Colletotrichum gloeosporioides* were evaluated *in vitro*. The new compounds were as active as avenaciolide in the tested conditions, while the synthetic precursors were inactive. The preparation and characterization of 15 new synthetic intermediates are also described.

Introduction. – The control of fungal diseases on plants often requires the use of fungicides, and there is a continuous need for new classes of antifungal agents due to the development of resistant strains. Avenaciolide (*Fig.*) is a naturally occurring antifungal bis-γ-lactone isolated from *Aspergillus avenaceus* [1]. Its structure was established by total synthesis and crystallographic studies [2][3]. Avenaciolide has also antibacterial action [1], inhibits the transport of glutamate in rat liver mitochondria [4], and interferes with the ability of ADP to stimulate the rate of glutamate oxidation [5]. Due to all these important biological activities combined with an interesting bicyclic structure, several synthetic approaches to avenaciolide have been published [6][7].

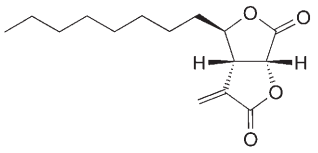


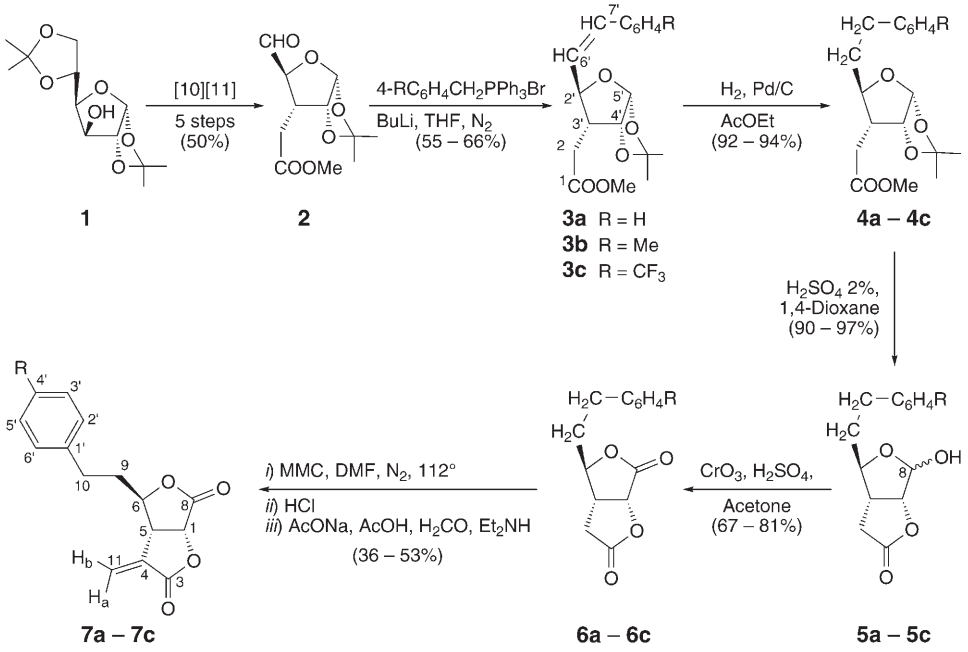
Figure. *Chemical structure of avenaciolide*

We have previously described the preparation of three aliphatic avenaciolide analogs [8], which were active against *Colletotrichum,* an important fungal genus that causes the plant disease known as anthracnose. *Colletotrichum* has a wide host range

including vegetables, field and forage crops, fruit trees, and ornamentals. Here, we describe the synthesis, characterization, and antifungal-activity evaluation against *Colletotrichum gloeosporioides* of three new avenaciolide analogs containing aromatic substituents. A large number of natural products containing an α-methylidene-γ-lactone group show a wide range of biological activities [9]. To investigate the importance of the O=CC=CH_2 system for the antifungal activity of avenaciolide and related compounds, the saturated synthetic precursors were also tested.

Results and Discussion. – *Syntheses of Bis-γ-lactones.* As the use of a carbohydrate as starting material [2] would enable us to prepare enantiomerically pure avenaciolide analogs, the synthetic approach shown in the *Scheme* was used for the preparation of compounds **7a**–**7c**, which bear aromatic groups in the side chain. These compounds and all the new synthetic intermediates (*i.e.*, compounds **3**–**6**; *Scheme*) were characterized by elemental analyses, and IR and NMR spectroscopy. The analyses of the NMR spectra were supported by DEPT, COSY, HMBC, and HSQC experiments.

Scheme



The aldehyde intermediate **2** was prepared from diacetone-D-glucose **1** as described in [10][11]. The different side chains were introduced by the *Wittig* reaction of the aldehyde **2** and the phosphonium ylides prepared *in situ* from the *Wittig* salts and BuLi, to yielding mixtures of (*Z*/*E*)-isomers **3a**–**3c**. The *Scheme* displays the arbitrary numbering of the C-atoms used for the NMR-signals attributions. The signals of the olefinic C(6′) and C(7′) of the major (*Z*)-isomers were observed at *ca.* 127 and 136 ppm, respectively, in the ^{13}C-NMR spectra of compounds **3a**–**3c**. Along with these signals,

other less intense ones were indicative of the presence of the (*E*)-isomers as minor components. The coupling constant (*J*) of *ca.* 11 Hz for the olefinic H-atoms H–C(6′) and H–C(7′) observed in the ^{1}H-NMR spectra of these compounds confirmed the (*Z*)-configuration for the main component. The observation of the expected signals in the aromatic range (7.1–7.6 ppm) confirmed the presence of the aromatic groups in the structures of **3a**–**3c**. It was not necessary to separate the (*Z*/*E*)-isomers to follow the synthetic approach chosen.

The hydrogenation of **3a**–**3c** yielded the compounds **4a**–**4c**, respectively, which, in the ^{13}C-NMR spectra, showed the two CH_2 signals at *ca.* 34 and 32 ppm, attributed to C(6′) and C(7′) in accordance with the correlations observed in their HSQC contour maps (H–C(6′) at 1.6–1.9 ppm and H–C(7′) at 2.6–2.9 ppm). Further, the correlation (^3J) observed between the aromatic C(2″) and C(6″) with the H–C(7′) signals in the HMBC contour map confirmed such attributions. The HMBC also showed a correlation (^2J) of H–C(7′) signals to C(1″), while the H–C(6′) signals were correlated only to this aromatic ^{13}C signal (^3J(H–C(6′),C(1″)).

The first lactone ring was closed by the reaction of the esters **4a**–**4c** with H_2SO_4 (2%) in 1,4-dioxane under reflux (*Scheme*). The several duplicated characteristic signal groups in the NMR spectra of **5a**–**5c** indicated the formation of mixtures of C(8) epimers. For example, two broad signals were observed for the OH groups (at *ca.* 3.7 and 3.4 ppm for OH in α- and β-positions, at a 1:2 proportion, resp.) in the ^{1}H-NMR spectrum of **5a**. These signals were obscured by other signals in the spectra of **5b** and **5c**, but the integration curves were indicative of their presence. The identity of the major product was established by the comparison of the H–C(8) signals, which appeared as *singlet*s at *ca.* 5.6 ppm for the epimers with the OH group in β-position, and as *doublet*s (J(8,1)$\approx$4 Hz) at 5.5 ppm for the minor α-isomers. The correlated C(8) signals were observed at *ca.* 101 and 96 ppm (less intense), respectively, in the ^{13}C-NMR spectra of the mixtures **5a**–**5c**. The C=O stretching band was observed in the IR spectra of **5a** and **5b** as a broad band centered at 1781 and 1755 cm^{-1}, respectively. Interestingly, two distinct bands were observed in the spectrum of **5c** at 1780 and 1746 cm^{-1} (less intense). The most intense band was attributed to the major product, with the OH group in β-position. The lower wave number observed for the α-isomer is probably due to intramolecular H-bonding with the O-atom at C(3).

The *Jones* oxidation of the compounds **5a**–**5c** yielded the bis-γ-lactones **6a**–**6c**, respectively. In the IR spectra of **6a** and **6b**, two distinct $\tilde{\nu}$(C=O) absorptions could be observed (at *ca.* 1775 and 1795 cm^{-1}), while the spectrum of **6c** showed a broad band centered at 1779 cm^{-1}. The simplification of the H–C(1) signal multiplicity in the ^{1}H-NMR spectra of these compounds (*doublet*s at *ca.* 5.01 ppm, with J(1,5)$\approx$8 Hz) and the observation of the two C=O signals in their ^{13}C-NMR spectra (C(8) at *ca.* 170 and C(3) at *ca.* 173 ppm) confirmed the oxidation at C(8). The attributions of the C=O signals were supported by the HMBC contour maps which showed ^3J correlations between C(8) and H–C(6) signals, and ^2J correlations between C(3) and H_a–C(4) and H_b–C(4) signals.

The exocyclic methylene group was introduced into the bis-γ-lactone skeleton using the methodology described by *Parker* and *Johnson* [12]. The reaction of **6a**–**6c** with magnesium methyl carbonate (MMC; *Scheme*), followed by the addition of HCl, yielded the carboxylic acid intermediates, which were not isolated. To the crude

products (yellow oils) was added a mixture of AcONa, AcOH, CH_2O, H_2O, and Et_2NH, to yield the avenaciolide analogs **7a**–**7c**. The IR spectra of **7a**–**7c** showed additional bands due to the additional C=C stretching vibration (at *ca.* 1665 cm^{-1}). The spectra of compounds **7a** and **7c** showed a broad band in the C=O region of the γ-lactones, and, in the spectrum of **7b**, two well-resolved bands were observed at 1791 and 1760 cm^{-1}, which can be attributed to C(8)=O and C(3)=O, respectively. No changes were observed in the C(8)=O signals in the ^{13}C-NMR spectra of compounds **7a**–**7c**, when compared to the spectra of the parent bis-lactones. On the other hand, the introduction of the methylidene group α to C(3) caused the expected shift on this C=O signal from *ca.* 173 to 167 ppm. The HMBC contour maps for **7a**–**7c** showed ^3J correlations between C(3) and H_a–C(11) and H_b–C(11) signals, and ^4J correlations between C(8) and H_a–C(9) and H_b–C(9) signals, confirming the C=O signal attributions. The expected change in the chemical shifts of C(4) signals from *ca.* 33 ppm in the ^{13}C-NMR spectra of the parent bis-γ-lactones to *ca.* 134 ppm confirmed the formation of compounds **7a**–**7c**. As in the ^{1}H-NMR spectrum of avenaciolide [13], the signals of the vinylic H_a–C(11) and H_b–C(11) of the analogs **7a**–**7c** appeared as two *doublet*s at *ca.* 6.4 and 5.8 ppm in their spectra. The correlated C-signals (HSQC) were observed at *ca.* 126.5 ppm in the ^{13}C-NMR spectra of **7a**–**7c**. The COSY contour maps showed a ^4J coupling between the H–C(11) signals and H–C(5), which is also coupled to H–C(1) and H–C(6). The remaining signals were in very good agreement with the proposed structures.

Antifungal Screening. The bis-γ-lactones **6a**–**6c** and **7a**–**7c**, and avenaciolide were tested for their capacity to inhibit the growth of the phytopathogenic fungi *Colletotrichum gloeosporioides*. The test methodology was designed in order to allow the use of very low amounts of substances and to provide a fast way to evaluate the antifungal potential of the compounds. The commercial fungicide *Folicur* of the triazole group of fungicides used as a positive control is employed on the control of *C. gloeosporioides* in several cultures such as citrus, mango, papaya, guava, passion fruit, and grapes. It contains tebuconazole (200 g l^{-1}) as active ingredient and, under the test conditions, gives good responses at the very low dose employed (inhibition hale of *ca.* 4 cm in 48 h). Paper discs (6 mm) were dipped into the solutions of the compounds at 1000 ppm. The discs were removed from the solutions and, after evaporation of the solvent, they were placed in the center of *Petri* dishes containing *C. gloeosporioides* conidia mixed with the BDA media. After 48 h of incubation at 25°, compounds **7a**–**7c** and avenaciolide presented inhibition hales (limited by the region where a dense and filamentous growth could be observed with the aid of a stereoscopic microscope) of 39–47% of the diameter of the positive control (*Folicur*). It has been shown that other α-methylidene-γ-lactones react rapidly with enzymes to form stable adducts, explaining at least in part their biological activity [14][15]. Compounds **6a**–**6c** were totally inactive under the test conditions. These results indicate that not only the bis-γ-lactone skeleton is important for the antifungal activity of these compounds, but also it depends on the presence of the exocyclic C=C bond probably due to a *Michael*-type addition reaction with the fungi enzymes.

The small differences on the aromatic groups of compounds **7a**–**7c** did not lead to great differences in their antifungal activities in the *in vitro* test employed. In fact, the results for **7b** and **7c** (1.5 cm, 39% inhibition) could not be differentiated by using the

Scott–Knott test at 5% of probability [16]. By a similar methodology, but at the concentration of 100 ppm and with a different positive control (benomyl), it was observed for aliphatic analogs containing pentyl, hexyl, and heptyl groups [8] that the increase in the C-chain length seems to increase the activity (39, 40, and 46%, resp.). The same feature was not observed in these tests, and compound **7a** was found more active than **7b** and **7c**. The activity of **7a** (47%) was in the same range of that presented by avenaciolide (45%), showing that the increase of the C-chain length is not the only factor influencing the antifungal activity of these bis-lactones. It is noteworthy that another very interesting result could be observed in these tests: although the tebuconazole causes a much broader inhibition hale, the *Petri* dishes containing the compounds **7a**–**7c** or avenaciolide showed a completely transparent hale, while the hale of the control was homogeneously opaque.

To further investigate the biological activities of this class of compounds and to evaluate their applicability as agrochemicals, other analogues are being prepared, and tests of different methodologies will be carried out.

We thank the Brazilian agencies *FAPEMIG* (CEX-860/02) for financial support, and *CNPq* and *CAPES* for students' grants.

Experimental Part

General. Diacetone-D-glucose **1** and the aryl bromides ($PhCH_2Br$, 4-methylbenzyl bromide, and 4-trifluoromethyl bromide) were purchased from *Aldrich*. Aldehyde **2** was prepared from **1** as described in [10][11]. Avenaciolide was prepared from **2** as described in [2]. Solvents were distilled before use and dried according to standard procedures. Optical rotations: *Bellingham + Stanley* model *D* polarimeter. IR Spectra: *Perkin Elmer Paragon 1000* spectrometer (4000–400 cm^{-1}) in KBr (when solids) or as thin films on NaCl plates (when oils). NMR Spectra: in $CDCl_3$ with a *Bruker DRX-400 AVANCE* or a *Bruker DPX-200 AVANCE* spectrometer; chemical shifts δ in ppm rel. to TMS as internal standard, and coupling constants *J* in Hz; the assignments of signals in NMR spectra of the new compounds **4**–**7** were supported by 2D experiments (COSY, HSQC, and HMBC contour maps). Microanalyses were performed with a *Perkin-Elmer 2400* elemental analyzer.

Preparation of **3a**–**3c**. The appropriate aryl bromide ($PhCH_2Br$ (1.71 g) or 4-methylbenzyl bromide (1.76 g), or 4-trifluoromethylbenzyl bromide (2.05 g)) was added to a stirring soln. of Ph_3P (2.62 g, 3.55 g and 3.20 g, resp.) in dry benzene (4 ml) at r.t. under N_2. The mixture was stirred under reflux for 2 h. The product was filtered, washed with Et_2O and dried under reduced pressure yielding the *Wittig* salts as white solids ((benzyl)(triphenyl)phosphonium bromide (**a**, 4.20 g, 97%), (4-methylbenzyl)(triphenyl)-phosphonium bromide (**b**, 3.83 g, 90%) and [4-(trifluoromethyl)benzyl](triphenyl)phosphonium bromide (**c**, 4.08 g, 95%)). BuLi (15% in hexane; 2.8, 2.7, or 2.6 ml for **a**, **b**, or **c**, resp.) was added to a stirring soln. of the *Wittig* salt (**a**: 2.64 g, 6.1 mmol; **b**: 3.21 g, 6.4 mmol; or **c**: 2.73 g, 6.1 mmol) in dry THF (20 ml) under N_2. The mixture was stirred for 10 min prior to the addition of a soln. of **2** (1.30 g, 5.3 mmol) in dry THF (7 ml). After 18 h stirring at r.t., the mixture was concentrated *i.v.*, H_2O (25 ml) was added, and extractions were performed with Et_2O (5×25 ml). The org. phase was dried (Na_2SO_4), concentrated, and submitted to CC (SiO_2; hexane/AcOEt 3:1) to yield the mixture of isomers **3a** (0.93 g, 55%), **3b** 0.99 g, 56%), or **3c** (1.36 g, 66%).

*Methyl 2-{(2*R*,3*R*,4*R*,5*R*)-2,3,4,5-Tetrahydro-4,5-(isopropylidenedioxy)-2-[(*Z*)-2-phenylethenyl]-furan-3-yl}acetate and Methyl 2-{(2*R*,3*R*,4*R*,5*R*)-2,3,4,5-Tetrahydro-4,5-(isopropylidenedioxy)-2-[(*E*)-2-phenylethenyl]furan-3-yl}acetate* (**3a**). White crystals. R_f (hexane/AcOEt 3:1) 0.51. M.p. 73.7–76.2° (hexane/AcOEt 3:1). IR (KBr): 2987, 2942, 1735, 1493, 1437, 1385, 1330, 1209, 1169, 1021, 873, 799, 705. ^{1}H-NMR ($CDCl_3$, 400 MHz)[1]): 1.31 (*s*, Me); 1.34 (*s*, Me*); 1.42 (*s*, Me); 1.54 (*s*, Me*); 2.21–2.27 (*m*,

[1]) The signals marked '*' are due to the (*E*)-isomer. All the others refer to the major (*Z*)-isomer.

H_a–C(2), H–C(3′), H–C(3′*)); 2.38 (*dd*, ^2J(2a*,2b*) = 17.4, ^3J(2a*,3′*) = 4.7, H_a–C(2*)); 2.53 (*dd*, ^2J(2b,2a) = 17.6, ^3J(2b,3′) = 11.2, H_b–C(2)); 2.69–2.75 (*m*, H_b–C(2*)); 3.63 (*s*, MeO*); 3.67 (*s*, MeO); 4.31 (*dd*, ^3J(2′*,3′*) = 10.7, ^3J(2′*,6′*) = 8.0, H–C(2′*)); 4.63 (*dd*, ^3J(2′,3′) = 9.8, ^3J(2′,6′) = 9.8, H–C(2′)); 4.79 (*dd*, ^3J(4′,5′) = 3.9, ^3J(4′,3′) = 3.9, H–C(4′)); 4.82 (*dd*, ^3J(4′*,5′*) = 4.1, ^3J(4′*,3′*) = 4.1, H–C(4′*)); 5.57 (*dd*, ^3J(6′,7′) = 11.4, ^3J(6′,2′) = 9.8, H–C(6′)); 5.90 (*d*, ^3J(5′,4′) = 3.9, H–C(5′)); 6.07 (*dd*, ^3J(6′*,7′*) = 15.8, ^3J(6′*,2′*) = 8.4, H–C(6′*)); 6.65 (*d*, ^3J(7′*,6′*) = 15.6, H–C(7′*)); 6.81 (*d*, ^3J(7′,6′) = 11.4, H–C(7′)); 7.26–7.38 (*m*, H–C(2″), H–C(3″), H–C(4″), H–C(5″), H–C(6″), H–C(2″*), H–C(3″*), H–C(4″*), H–C(5″*), H–C(6″*)). ^{13}C-NMR ($CDCl_3$, 100 MHz)[1]): 26.3 (2 Me*); 26.5 (2 Me); 29.1 (C(2)); 29.4 (C(2*)); 45.9 (C(3′*)); 46.6 (C(3′)); 51.7 (MeO*); 51.8 (MeO); 76.0 (C(2′)); 80.8 (C(4′)); 81.9 (C(2′*)); 105.0 (C(5′)); 111.6 (Me_2C); 111.7 (Me_2C*); 126.1 (C(6′*)); 126.7 (C(2″*), C(6″*)); 127.7 (C(6′), C(4″)); 128.1 (C(4″*)); 128.3 (C(3″), C(5″)); 128.6 (C(3″*), C(5″*)); 128.8 (C(2″), C(6″)); 134.5 (C(7′*)); 136.0 (C(1″)); 136.1 (C(1″*)); 136.8 (C(7′)); 172.0 (C(1*)); 172.5 (C(1)). Anal. calc. for $C_{18}H_{22}O_5$ (318.37): C 67.91, H 6.97; found: C 68.14, H 7.01.

*Methyl 2-{(2*R*,3*R*,4*R*,5*R*)-2,3,4,5-Tetrahydro-4,5-(isopropylidenedioxy)-2-[(*Z*)-2-(4-methylphenyl)-ethenyl]furan-3-yl}acetate and Methyl 2-{(2*R*,3*R*,4*R*,5*R*)-4,5-isopropylidenedioxy-[(*E*)-2-(4-methylphenyl)ethenyl]furan-3-yl}acetate* (**3b**). White crystals. R_f (hexane/AcOEt 3:1) 0.54. M.p. 109.5–112.5° (hexane/AcOEt 3:1). IR (KBr): 2989, 2957, 1735, 1511, 1435, 1373, 1328, 1209, 1169, 1023, 965, 872, 835. ^{1}H-NMR ($CDCl_3$, 200 MHz)[1]): 1.32 (*s*, Me); 1.34 (*s*, Me*); 1.45 (*s*, Me); 1.54 (*s*, Me*); 2.10–2.35 (*m*, H_a–C(2), H–C(3′), H_a–C(2*), H–C(3′*)); 2.35 (*s*, Me–C(4″)); 2.54 (*dd*, ^2J(2b,2a) = 17.4, ^3J(2b,3′) = 11.1, H_b–C(2)); 2.68 (*dd*, ^2J(2b*,2a*) = 16.5, ^3J(2b*,3′*) = 9.9, H_b–C(2*)); 3.63 (*s*, MeO*); 3.67 (*s*, MeO); 4.25–4.34 (*m*, H–C(2′*)); 4.65 (*dd*, ^3J(2′,3′) = 9.7, ^3J(2′,6′) = 9.7, H–C(2′)); 4.77–4.84 (*m*, H–C(4′*)); 4.79 (*dd*, ^3J(4′,5′) = 3.8, ^3J(4′,3′) = 3.8, H–C(4′)); 5.51 (*dd*, ^3J(6′,7′) = 11.2, ^3J(6′,2′) = 9.6, H–C(6′)); 5.90 (*d*, ^3J(5′,4′) = 3.8, H–C(5′)); 6.00 (*dd*, ^3J(6′*,7′*) = 15.9, ^3J(6′*,2′*) = 8.2, H–C(6′*)); 6.62 (*d*, ^3J(7′*,6′*) = 15.9, H–C(7′*)); 6.77 (*d*, ^3J(7′,6′) = 11.2, H–C(7′)); 7.10–7.17 (*m*, H–C(3″), H–C(5″), H–C(3″*), H–C(5″*)); 7.25–7.29 (*m*, H–C(2″), H–C(6″), H–C(2″*), H–C(6″*)). ^{13}C-NMR ($CDCl_3$, 50 MHz)[1]): 21.2 (Me–C(4″)); 26.3 (Me*); 26.5 (Me); 26.6 (Me, Me*); 29.1 (C(2), C(2*)); 45.9 (C(3′*)); 46.6 (C(3′)); 51.7 (MeO, MeO*); 76.1 (C(2′)); 80.7 (C(4′*)); 80.8 (C(4′)); 82.1 (C(2′*)); 105.0 (C(5′), C(5′*)); 111.6 (Me_2C); 111.7 (Me_2C*); 124.9 (C(6′*)); 126.6 (C(2″*), C(6″*)); 126.9 (C(6′)); 128.8 (C(2″), C(6″)); 129.0 (C(3″), C(5″)); 129.3 (C(3″*), C(5″*)); 133.1 (C(1″)); 133.3 (C(1″*)); 134.5 (C(7′*)); 136.8 (C(7′)); 137.6 (C(4″)); 138.1 (C(4″*)); 171.2 (C(1*)); 172.5 (C(1)). Anal. calc. for $C_{19}H_{24}O_5$ (332.40): C 68.66, H 7.28; found: C 69.13, H 6.84.

*Methyl 2-((2*R*,3*R*,4*R*,5*R*)-2,3,4,5-Tetrahydro-4′,5′-(isopropylidenedioxy)-2-{(*Z*)-2-[4-(trifluoromethyl)phenyl]ethenyl}furan-3-yl)acetate and Methyl 2-(2*R*,3*R*,4*R*,5*R*)-2,2,4,5-Tetrahydro-4,5-(isopropylidenedioxy)-2-{(*E*)-2-(4-[4-(trifluoromethyl)phenyl]ethenyl}furan-3-yl)acetate* (**3c**). Colorless oil. R_f (hexane/AcOEt 3:1) 0.46. IR (film): 2989, 2955, 1739, 1617, 1439, 1383, 1375, 1326, 1217, 1166, 1127, 1067, 1017, 864. ^{1}H-NMR ($CDCl_3$, 400 MHz)[1]): 1.32 (*s*, Me); 1.35 (*s*, Me*); 1.42 (*s*, Me); 1.55 (*s*, Me*); 2.19–2.31 (*m*, H_a–C(2), H–C(3′), H–C(3′*)); 2.38 (*dd*, ^2J(2a*,2b*) = 16.8, ^3J(2a*,3′*) = 4.6, H_a–C(2*)); 2.54 (*dd*, ^2J(2b,2a) = 16.1, ^3J(2b,3′) = 9.5, H_b–C(2)); 2.71 (*dd*, ^2J(2b*,2a*) = 16.8, ^3J(2b*,3′*) = 9.9, H_b–C(2*)); 3.64 (*s*, MeO*); 3.68 (*s*, MeO); 4.34 (*dd*, ^3J(2′*,3′*) = 10.2, ^3J(2′*,6′*) = 8.5, H–C(2′*)); 4.53 (*dd*, ^3J(2′,3′) = 9.7, ^3J(2′,6′) = 9.7, H–C(2′)); 4.79 (*dd*, ^3J(4′,5′) = 3.9, ^3J(4′,3′) = 3.9, H–C(4′)); 4.84 (*dd*, ^3J(4′*,5′*) = 4.1, ^3J(4′*,3′*) = 4.1, H–C(4′*)); 5.69 (*dd*, ^3J(6′,7′) = 11.4, ^3J(6′,2′) = 9.7, H–C(6′)); 5.90 (*d*, ^3J(5′,4′) = 3.9, H–C(5′)); 5.90–5.92 (*m*, H–C(5′*)); 6.18 (*dd*, ^3J(6′*,7′*) = 15.9, ^3J(6′*,2′*) = 7.8, H–C(6′*)); 6.69 (*d*, ^3J(7′*,6′*) = 15.9, H–C(7′*)); 6.82 (*d*, ^3J(7′,6′) = 11.4, H–C(7′)); 7.46–7.50 (*m*, H–C(3″), H–C(5″), H–C(3″*), H–C(5″*)); 7.56–7.61 (*m*, H–C(2″), H–C(6″), H–C(2″*), H–C(6″*)). ^{13}C-NMR ($CDCl_3$, 100 MHz)[1]): 26.3 (Me*); 26.5 (Me); 26.6 (Me, Me*); 29.1 (C(2)); 29.2 (C(2*)); 45.9 (C(3′*)); 46.6 (C(3′)); 51.8 (MeO, MeO*); 75.7 (C(2′)); 80.8 (C(4′), C(4′*)); 81.5 (C(2′*)); 105.0 (C(5′), C(5′*)); 111.8 (Me_2C, Me_2C*); 125.3 (*q*, ^3J(C,F) = 3.8, C(3″), C(5″)); 125.6 (*q*, ^3J(C,F) = 3.9, C(3″*), C(5″*)); 126.8 (C(6′)); 128.9 (C(6′*)); 129.1 (C(2″), C(6″)); 129.8 (C(2″*), C(6″*)); 129.8 [*q*, $^2J_{C,F}$ = 32.3, C(4″)); 132.7 (C(7′*)); 135.4 (C(7′)); 139.5 (C(1″)); 172.3 (C(1)). Anal. calc. for $C_{19}H_{21}F_3O_5$ (386.37): C 59.07, H 5.48; found: C 58.88, H 5.31.

Preparation of **4a**–**4c**. To a soln. of **3a** (0.87 g, 2.7 mmol), **3b** (0.87 g; 2.6 mmol), or **3c** (1.10 g; 2.8 mmol) in AcOEt (120 ml), 60 mg of Pd/C 10% were added. The suspension was shaken under H_2 for

20 h at r.t. The mixture was filtered, and the solvent was removed *i.v.* to yield the esters **4a** (0.81 g, 92%), **4b** (0.82 g, 94%), and **4c** (1.04 g, 94%), resp.

*Methyl 2-[(2*R*,3*R*,4*R*,5*R*)-2,3,4,5-Tetrahydro-4,5-(isopropylidenedioxy)-2-(2-(phenylethyl)furan-3-yl)acetate* (**4a**). White crystals. R_f (hexane/AcOEt 3:1) 0.55. M.p. 77.8–79.5° (hexane/AcOEt 3:1). $[\alpha]_D^{24} = +173.1$ ($c = 1.04$, CH_2Cl_2). IR (KBr): 3028, 2985, 2943, 2867, 1734, 1602, 1461, 1440, 1387, 1375, 1212, 1136, 1091, 1011, 871, 703. ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.32 (*s*, Me); 1.47 (*s*, Me); 1.67–1.77 (*m*, H_a–C(6′)); 1.82–1.90 (*m*, H_b–C(6′)); 2.04–2.13 (*m*, H–C(3′)); 2.28 (*dd*, ^2J(2a,2b) = 16.9, ^3J(2a,3′) = 4.3, H_a–C(2)); 2.58–2.72 (*m*, H_a–C(7′)); 2.61 (*dd*, ^2J(2b,2a) = 16.9, ^3J(2b,3′) = 10.2, H_b–C(2)); 2.84–2.91 (*m*, H_b–C(7′)); 3.69 (*s*, MeO); 3.76–3.81 (*m*, H–C(2′)); 4.76 (*t*, ^3J(4′,5′) = 4.0, ^3J(4′,3′) = 4.0, H–C(4′)); 5.85 (*d*, ^3J(5′,4′) = 4.0, H–C(5′)); 7.15–7.20 (*m*, H–C(3″), H–C(4″), H–C(5″)); 7.25–7.29 (*m*, H–C(2″), H–C(6″)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 26.4 (Me); 26.5 (Me); 29.5 (C(2)); 32.3 (C(7′)); 34.5 (C(6′)); 44.8 (C(3′)); 51.8 (MeO); 79.5 (C(2′)); 81.2 (C(4′)); 104.7 (C(5′)); 111.4 (CMe_2); 125.9 (C(4) of Ph); 128.4 (C(2), C(3), C(5), C(6) of Ph); 141.8 (C(1) of Ph); 172.6 (C(1)). Anal. calc. for $C_{18}H_{24}O_5$ (320.39): C 67.48, H 7.55; found: C 67.84, H 7.73.

*Methyl 2-{(2*R*,3*R*,4*R*,5*R*)-2,3,4,5-Tetrahydro-4,5-(isopropylidenedioxy)-2-[2-(4-methylphenyl)-ethyl]furan-3-yl}acetate* (**4b**). White crystals. R_f (hexane/AcOEt 3:1) 0.60. M.p. 56.7–58.0° (hexane/AcOEt 3:1). $[\alpha]_D^{21} = +225.5$ ($c = 1.02$, CH_2Cl_2). IR (KBr): 2985, 2943, 2859, 1737, 1514, 1434, 1373, 1335, 1255, 1211, 1140, 1017, 978, 879, 807, 532. ^{1}H-NMR ($CDCl_3$, 200 MHz): 1.32 (*s*, Me); 1.47 (*s*, Me); 1.60–1.92 (*m*, CH_2(6′)); 2.01–2.16 (*m*, H–C(3′)); 2.28 (*dd*, ^2J(2a,2b) = 16.9, ^3J(2a,3′) = 4.0, H_a–C(2)); 2.31 (*s*, Me–C(4) of C_6H_4); 2.60–2.71 (*m*, H_a–C(7′)); 2.63 (*dd*, ^2J(2b,2a) = 16.9, ^3J(2b,3′) = 10.3, H_b–C(2)); 2.76–2.91 (*m*, H_b–C(7′)); 3.69 (*s*, MeO); 3.73–3.84 (*m*, H–C(2′)); 4.76 (*t*, ^3J(4′,5′) = 4.0, ^3J(4′,3′) = 4.0, H–C(4′)); 5.84 (*d*, ^3J(5′,4′) = 4.0, H–C(5′)); 7.08 (br. *s*, H–C(2″), H–C(3″), H–C(5″), H–C(6″)). ^{13}C-NMR ($CDCl_3$, 50 MHz): 21.0 (Me–C(4) of C_6H_4); 26.4 (Me); 26.6 (Me); 29.5 (C(2)); 31.8 (C(7′)); 34.6 (C(6′)); 44.8 (C(3′)); 51.8 (MeO); 79.5 (C(2′)); 81.1 (C(4′)); 104.7 (C(5′)); 111.4 (Me_2C); 128.8 (C(2), C(6) of C_6H_4); 129.1 (C(3), C(5) of C_6H_4); 135.3 (C(4) of C_6H_4); 138.8 (C(1) of C_6H_4); 172.6 (C(1)). Anal. calc. for $C_{19}H_{26}O_5$ (334.41): C 68.24, H 7.84; found: C 68.64, H 7.74.

*Methyl 2-{(2*R*,3*R*,4*R*,5*R*)-2,3,4,5-Tetrahydro-4,5-(isopropylidenedioxy)-2-[2-[4-(trifluoromethyl)-phenyl]ethyl]furan-3-yl}acetate* (**4c**). White crystals. R_f (hexane/AcOEt 3:1) 0.49. M.p. 71.7–74.2° (hexane/AcOEt 3:1). $[\alpha]_D^{26} = +203.6$ ($c = 1.67$, CH_2Cl_2). IR (KBr): 2992, 2961, 2864, 1735, 1617, 1431, 1325, 1209, 1164, 1118, 1017, 895. ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.32 (*s*, Me); 1.46 (*s*, Me); 1.71–1.77 (*m*, H_a–C(6′)); 1.83–1.88 (*m*, H_b–C(6′)); 2.06–2.14 (*m*, H–C(3′)); 2.27 (*dd*, ^2J(2a,2b) = 16.9, ^3J(2a,3′) = 4.5, H_a–C(2)); 2.64 (*dd*, ^2J(2b,2a) = 16.9, ^3J(2b,3′) = 9.8, H_b–C(2)); 2.72–2.79 (*m*, H_a–C(7′)); 2.89–2.96 (*m*, H_b–C(7′)); 3.69 (*s*, MeO); 3.74–3.79 (*m*, H–C(2′)); 4.76 (*dd*, ^3J(4′,5′) = 4.0, ^3J(4′,3′) = 4.0, H–C(4′)); 5.84 (*d*, ^3J(5′,4′) = 4.0, H–C(5′)); 7.30 (*d*, ^3J(2″,3″) = 8.0, ^3J(6″,5″) = 8.0, H–C(2″), H–C(6″)); 7.53 (*d*, ^3J(3″,2″) = 8.0, ^3J(5″,6″) = 8.0, H–C(3″), H–C(5″)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 26.3 (Me); 26.5 (Me); 29.5 (C(2)); 32.0 (C(7′)); 34.0 (C(6′)); 44.7 (C(3′)); 51.7 (MeO); 79.2 (C(2′)); 81.2 (C(4′)); 104.6 (C(5′)); 111.5 (Me_2C), 125.3 (*q*, ^3J(C,F) = 3.7, C(3), C(5) of C_6H_4); 128.4 (*q*, ^2J(C,F) = 32.1, C(4) of C_6H_4); 128.7 (C(2), C(6) of C_6H_4); 145.9 (C(1) of C_6H_4); 172.5 (C(1)). Anal. calc. for $C_{19}H_{23}F_3O_5$ (388.38): C 58.76, H 5.97; found: C 58.87, H 6.13.

Preparation of **5a**–**5c**. To a stirring soln. of the esters **4a** (0.74 g, 2.3 mmol), **4b** (0.75 g, 2.2 mmol), or **4c** (0.94 g, 2.4 mmol) in 1,4-dioxane (35 ml), an aq. soln. of 2% H_2SO_4 (*v*/*v*; 16 ml) was added. The mixture was stirred under reflux for 3 h. The product was extracted with Et_2O (200 ml). The org. phase was washed with dist. H_2O (35 ml) and with sat. aq. $NaHCO_3$ soln. (35 ml), dried (Na_2SO_4), and concentrated *i.v.* The crude material was purified by CC (SiO_2; hexane/AcOEt 1:1) to yield the mixture of epimers **5a** (0.54 g, 94%), **5b** (0.57 g, 97%), and **5c** (0.69 g, 90%), resp.

*(1*R*,5*R*,6*R*,8*R*)- and (1*R*,5*R*,6*R*,8*S*)-8-Hydroxy-6-(2-phenylethyl)-2,7-dioxabicyclo[3.3.0]octan-3-one* (**5a**). Colorless oil. R_f (hexane/AcOEt 1:1) 0.43. IR (film): 3430, 3032, 2936, 2864, 1781, 1636, 1496, 1455, 1362, 1167, 1047, 702. ^{1}H-NMR ($CDCl_3$, 400 MHz)[2]): 1.88–2.00 (*m*, H_a–C(9), CH_2(9*)); 2.07–2.17 (*m*, H_b–C(9)); 2.40 (*dd*, ^2J(4a,4b) = 18.0, ^3J(4a,5) = 1.6, H_a–C(4)); 2.42–2.49 (*m*, H_a–C(4*)); 2.64–2.83 (*m*, CH_2(10), H_b–C(4*), H–C(5*), CH_2(10*)); 2.77 (*dd*, ^2J(4b,4a) = 18.0, ^3J(4b,5) = 9.2,

[2]) The signals marked '*' are due to the α-epimer. All the others refer to the major β-epimer (α/β 1:2).

H_b–C(4)); 2.88–2.94 (*m*, H–C(5)); 3.40 (br. *s*, OH); 3.70 (br. *s*, OH*); 3.94 (*ddd*, ^3J(6,5) = 8.2, ^3J(6,9a) = 5.2, ^3J(6,9b) = 5.2, H–C(6)); 3.98–4.03 (*m*, H–C(6*)); 4.87 (*d*, ^3J(1,5) = 6.3, H–C(1)); 4.87–4.90 (*m*, H–C(1*)); 5.53 (*d*, ^3J(8*,1*) = 4.0, H–C(8*)); 5.56 (*s*, H–C(8)); 7.17–7.31 (*m*, H–C(2′), H–C(3′), H–C(4′), H–C(5′), H–C(6′), H–C(2′*), H–C(3′*), H–C(4′*), H–C(5′*), H–C(6′*)). ^{13}C-NMR ($CDCl_3$, 100 MHz)[2]): 32.0 (C(10*)); 32.3 (C(10)); 33.3 (C(4*)); 33.9 (C(4)); 36.4 (C(9*)); 39.2 (C(9)); 42.1 (C(5*)); 42.6 (C(5)); 82.2 (C(1*)); 82.7 (C(6*)); 87.3 (C(6)); 88.3 (C(1)); 95.6 (C(8*)); 101.1 (C(8)); 126.2 (C(4′), C(4′*)); 128.3 (C(2′*), C(6′*)); 128.4 (C(2′), C(6′)); 128.6 (C(3′), C(5′), C(3′*), C(5′*)); 140.9 (C(1′*)); 141.0 (C(1′)); 175.7 (C(3)); 176.6 (C(3*)). Anal. calc. for $C_{14}H_{16}O_4$ (248.28): C 67.73, H 6.50; found: C 67.83, H 6.56.

*(1*R*,5*R*,6*R*,8*R*)- and (1*R*,5*R*,6*R*,8*S*)-8-Hydroxy-2,7-6-[2-(4-methylphenyl)ethyl)-dioxabicyclo[3.3.0]octan-3-one* (**5b**). White crystals. R_f (hexane/AcOEt 1:1) 0.47. M.p. 77.0–78.5° (hexane/AcOEt 1:1). IR (KBr): 3419, 3042, 2950, 2910, 2860, 1755, 1515, 1451, 1414, 1198, 1179, 1063, 1048, 973, 907, 802, 778. ^{1}H-NMR ($CDCl_3$, 200 MHz)[2]): 1.84–1.97 (*m*, H_a–C(9), CH_2(9*)); 2.00–2.19 (*m*, H_b–C(9)); 2.32 (*s*, Me, Me*); 2.35–2.48 (*m*, H_a–C(4), H_a–C(4*)); 2.69–2.89 (*m*, H_b–C(4), CH_2(10), H_b–C(4*), H–C(5*), CH_2(10*), OH); 2.84–2.95 (*m*, H–C(5), OH*); 3.93 (*ddd*, ^3J(6,5) = 8.1, ^3J(6,9a) = 5.2, ^3J(6,9b) = 5.2, H–C(6)); 3.96–4.04 (*m*, H–C(6*)); 4.87 (*d*, ^3J(1,5) = 6.2, H–C(1)); 4.86–4.91 (*m*, H–C(1*)); 5.53 (*d*, ^3J(8*,1*) = 4.1, H–C(8*)); 5.55 (*s*, H–C(8)); 7.04–7.13 (*m*, H–C(2′), H–C(3′), H–C(5′), H–C(6′), H–C(2′*), H–C(3′*), H–C(5′*), H–C(6′*)). ^{13}C-NMR ($CDCl_3$, 50 MHz)[2]): 20.9 (Me, Me*); 31.4 (C(10*)); 31.7 (C(10)); 33.3 (C(4*)); 33.9 (C(4)); 36.5 (C(9*)); 39.2 (C(9)); 42.0 (C(5*)); 42.5 (C(5)); 82.2 (C(1*)); 82.6 (C(6*)); 87.2 (C(6)); 88.3 (C(1)); 95.5 (C(8*)); 101.0 (C(8)); 128.2 (C(2′), C(6′), C(2′*), C(6′*)); 129.2 (C(3′), C(5′), C(3′*), C(5′*)); 135.6 (C(4′), C(4′*)); 137.8 (C(1′), C(1′*)); 175.7 (C(3)); 176.6 (C(3*)). Anal. calc. for $C_{15}H_{18}O_4$ (262.31): C 68.69, H 6.92; found: C 68.85, H 6.94.

*(1*R*,5*R*,6*R*,8*R*)- and (1*R*,5*R*,6*R*,8*S*)-8-Hydroxy-6-[2-(4-(trifluoromethyl)phenyl]ethyl]-2,7-dioxabicyclo[3.3.0]octan-3-one* (**5c**). White crystals. R_f (hexane/AcOEt 1:1) 0.42. M.p. 102.5–105.2° (hexane/AcOEt 1:1). IR (KBr): 3526, 3382, 2959, 2872, 1780, 1746, 1618, 1419, 1322, 1168, 1130, 1067, 1019, 963, 902, 631. ^{1}H-NMR ($CDCl_3$, 400 MHz)[2]): 1.90–1.99 (*m*, H_a–C(9), CH_2(9*)); 2.07–2.14 (*m*, H_b–C(9)); 2.42 (*dd*, ^2J(4a,4b) = 18.0, ^3J(4a,5) = 1.7, H_a–C(4)); 2.44–2.50 (*m*, H_a–C(4*)); 2.69–2.89 (*m*, CH_2(10), H_b–C(4*), H–C(5*), CH_2(10*), OH); 2.81 (*dd*, ^2J(4b,4a) = 18.0, ^3J(4b,5) = 9.1, H_b–C(4)); 2.89–2.95 (*m*, H–C(5)); 3.03 (br. *s*, OH*); 3.94 (*ddd*, ^3J(6,5) = 8.9, ^3J(6,9a) = 4.7, ^3J(6,9b) = 4.7, H–C(6)); 3.97–4.02 (*m*, H–C(6*)); 4.90 (*d*, ^3J(1,5) = 6.4, H–C(1)); 4.91 (*dd*, ^3J(1*,5*) = 8.5, ^3J(1*,8*) = 4.0, H–C(1*)); 5.55 (*d*, ^3J(8*,1*) = 4.0, H–C(8*)); 5.59 (*s*, H–C(8)); 7.30 (*d*, ^3J(2′,3′) = 8.0, ^3J(6′,5′) = 8.0, ^3J(2′*,3′*) = 8.0, ^3J(6′*,5′*) = 8.0, H–C(2′), H–C(6′), H–C(2′*), H–C(6′*)); 7.53 (*d*, ^3J(3′,2′) = 8.0, ^3J(5′,6′) = 8.0, ^3J(3′*,2′*) = 8.0, ^3J(5′*,6′*) = 8.0, H–C(3′), H–C(5′), H–C(3′*), H–C(5′*)). ^{13}C-NMR ($CDCl_3$, 100 MHz)[2]): 32.0 (C(10*)); 32.4 (C(10)); 33.4 (C(4*)); 34.1 (C(4)); 36.3 (C(9*)); 39.1 (C(9)); 42.3 (C(5*)); 42.9 (C(5)); 82.3 (C(1*)); 82.5 (C(6*)); 87.3 (C(6)); 88.4 (C(1)); 95.9 (C(8*)); 101.3 (C(8)); 125.6 (*q*, ^3J(C,F) = 3.6, C(3′), C(5′), C(3′*), C(5′*)); 128.8 (C(2′), C(6′)); 128.8 (*q*, ^2J(C,F) = 33.0, C(4′), C(4′*)); 128.9 (C(2′*), C(6′*)); 145.2 (C(1′*)); 145.4 (C(1′)); 175.6 (C(3)); 176.3 (C(3*)). Anal. calc. for $C_{15}H_{15}F_3O_4$ (316.28): C 56.96, H 4.78; found: C 57.11, H 4.96.

Preparation of **6a**–**6c**. The *Jones* reagent was prepared with CrO_3 (2.67 g), concentrated H_2SO_4 (2.3 ml), and distilled H_2O (up to 10.0 ml). A portion of of this soln. (1.4 ml) was added to a stirring soln. of the compounds **5a** (0.45 g, 1.8 mmol), **5b** (0.50 g, 1.9 mmol), or **5c** (0.59 g, 1.9 mmol) in acetone (25 ml). After 5 min at r.t., another portion of 1.4 ml of the *Jones* reagent was added, and the mixture was stirred for further 15 min, prior to the addition of MeOH (15 ml). Distilled H_2O (35 ml) was added, and the product was extracted with Et_2O (4 × 30 ml). The org. phase was washed with aq. $NaHCO_3$ soln. (30 ml), dried (Na_2SO_4), and concentrated *i.v.* to yield the bis-γ-lactones **6a** (0.36 g, 81%), **6b** (0.33 g, 67%), and **6c** (0.41 g, 70%), resp.

*(1*R*,5*R*,6*R*)-6-(2-Phenylethyl)-2,7-dioxabicyclo[3.3.0]octan-3,8-dione* (**6a**). White crystals. R_f (hexane/AcOEt 1:1) 0.49. M.p. 83.3–85.9° (hexane/AcOEt 1:1). $[\alpha]_D^{24} = +133.8$ (*c* = 1.39, CH_2Cl_2). IR (KBr): 3080, 3026, 2932, 2866, 1794, 1774, 1602, 1455, 1423, 1311, 1249, 1221, 1169, 1132, 1022, 1000, 931, 753. ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.97–2.12 (*m*, CH_2(9)); 2.47 (*dd*, ^2J(4a,4b) = 18.3, ^3J(4a,5) = 4.2, H_a–C(4)); 2.71–2.78 (*m*, H_a–C(10)); 2.82–2.89 (*m*, H_b–C(10)); 2.88 (*dd*, ^2J(4b,4a) = 18.3, ^3J(4b,5) = 9.5, H_b–C(4)); 3.00–3.07 (*m*, H–C(5)); 4.31 (*dt*, ^3J(6,5) = 8.2, ^3J(6,9) = 4.9, H–C(6)); 5.01 (*d*, ^3J(1,5) = 7.9, H–C(1)); 7.18–7.33 (*m*, H–C(2′), H–C(3′), H–C(4′), H–C(5′), H–C(6′)). ^{13}C-NMR ($CDCl_3$,

100 MHz): 31.3 (C(10)); 32.7 (C(4)); 37.2 (C(9)); 40.2 (C(5)); 76.9 (C(1)); 83.8 (C(6)); 126.6 (C(4′)); 128.4 (C(2′), C(6′)); 128.8 (C(3′), C(5′)); 139.6 (C(1′)); 169.8 (C(8)); 173.6 (C(3)). Anal. calc. for $C_{14}H_{14}O_4$ (246.26): C 68.28, H 5.73; found: C 68.36, H 5.73.

*(1*R*,5*R*,6*R*)-6-[2-(4-Methylphenyl)ethyl]-2,7-dioxabicyclo[3.3.0]octan-3,8-dione* (**6b**). White crystals. R_f (hexane/AcOEt 1:1) 0.51. M.p. 129.4–130.5° (hexane/AcOEt 1:1). $[\alpha]_D^{21} = +218.5$ ($c = 1.08$, CH_2Cl_2). IR (KBr): 3046, 2926, 2866, 1798, 1777, 1517, 1415, 1363, 1312, 1251, 1217, 1204, 1149, 1066, 1003, 986, 812, 722. ^{1}H-NMR ($CDCl_3$, 200 MHz): 1.96–2.09 (*m*, CH_2(9)); 2.33 (*s*, Me); 2.47 (*dd*, ^2J(4a,4b) = 18.0, ^3J(4a,5) = 3.9, H_a–C(4)); 2.63–2.83 (*m*, CH_2(10)); 2.88 (*dd*, ^2J(4b,4a) = 18.0, ^3J(4b,5) = 9.4, H_b–C(4)); 2.97–3.10 (*m*, H–C(5)); 4.30 (*dt*, ^3J(6,5) = 7.7, ^3J(6,9b) = 5.2, H–C(6)); 5.01 (*d*, ^3J(1,5) = 7.7, H–C(1)); 7.05–7.15 (*m*, H–C(2′), H–C(3′), H–C(5′), H–C(6′)). ^{13}C-NMR ($CDCl_3$, 50 MHz): 21.0 (Me); 30.8 (C(10)); 32.7 (C(4)); 37.2 (C(9)); 40.1 (C(5)); 76.9 (C(1)); 83.8 (C(6)); 128.3 (C(2′), C(6′)); 129.5 (C(3′), C(5′)); 136.2 (C(4′)); 136.5 (C(1′)); 169.8 (C(8)); 173.6 (C(3)). Anal. calc. for $C_{15}H_{16}O_4$ (260.29): C 69.22, H 6.20; found: C 69.59, H 5.86.

*(1*R*,5*R*,6*R*)-6-{2-[4-(Trifluoromethyl)phenyl]ethyl}-2,7-dioxabicyclo[3.3.0]octan-3,8-dione* (**6c**). White crystals. R_f (hexane/AcOEt 1:1) 0.50. M.p. 114.5–117.1° (hexane/AcOEt 1:1). $[\alpha]_D^{26} = +146.9$ ($c = 1.77$, CH_2Cl_2). IR (KBr): 3048, 2964, 2922, 2872, 2844, 1779, 1617, 1420, 1324, 1213, 1119, 1065, 1020, 935, 847, 600. ^{1}H-NMR ($CDCl_3$, 400 MHz): 2.03–2.08 (*m*, CH_2(9)); 2.51 (*dd*, ^2J(4a,4b) = 18.3, ^3J(4a,5) = 3.9, H_a–C(4)); 2.78–2.86 (*m*, H_a–C(10)); 2.91–2.98 (*m*, H_b–C(10)); 2.92 (*dd*, ^2J(4b,4a) = 18.3, ^3J(4b,5) = 9.4, H_b–C(4)); 3.02–3.07 (*m*, H–C(5)); 4.28–4.32 (*m*, H–C(6)); 5.03 (*d*, ^3J(1,5) = 7.8, H–C(1)); 7.31 (*d*, ^3J(2′,3′) = 8.1, ^3J(6′,5′) = 8.1, H–C(2′), H–C(6′)); 7.58 (*d*, ^3J(3′,2′) = 8.1, ^3J(5′,6′) = 8.1, H–C(3′), H–C(5′)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 31.3 (C(10)); 32.6 (C(4)); 37.1 (C(9)); 40.3 (C(5)); 76.7 (C(1)); 83.3 (C(6)); 125.8 (*q*, ^3J(C,F) = 4.0, C(3′), C(5′)); 128.8 (C(2′), C(6′)); 129.2 (*q*, ^2J(C,F) = 32.1, C(4′)); 143.7 (*q*, ^5J(C,F) = 1.3, C(1′)); 169.4 (C(8)); 173.1 (C(3)). Anal. calc. for $C_{15}H_{13}F_3O_4$ (314.26): C 57.33, H 4.17; found: C 57.26, H 4.43.

Preparation of **7a**–**7c**. A soln. of magnesium methyl carbonate (MMC; 2.0M in DMF; 4.9, 5.2, and 4.7 ml, resp.) was added to the bis-γ-lactone **7a** (0.22 g, 0.89 mmol), **7b** (0.25 g, 0.96 mmol), or **7c** (0.27 g, 0.86 mmol) under N_2. The mixture was stirred at 112° for 5 h and then was poured into an ice-cold mixture of 6M HCl/Et_2O 5:1 (24 ml), and stirred in order to dissolve the precipitate formed. The phases were separated, and extractions with Et_2O were performed (2 × 10 ml). The combined org. phases were washed with sat. aq. NaCl soln. (15 ml), dried (Na_2SO_4), and concentrated *i.v.* To the yellow oil thus obtained, it was added a previously prepared mixture of AcONa (91, 96 and 87 mg for **7a**, **7b**, and **7c**, resp.), AcOH (3.4 ml), formalin (2.5 ml), and Et_2NH (1.1 ml). The mixture was vigorously shaken for 1 min and then heated on a steam bath for 5 min, cooled, and poured into H_2O (27 ml) and Et_2O (17 ml). The org. phase was washed with H_2O (12 ml) and sat. aq. $NaHCO_3$ soln. (12 ml), and dried (Na_2SO_4) and concentrated *i.v.* The white solid obtained was purified by CC (SiO_2, hexane/AcOEt 1:1 to yield compounds **7a** (0,12 g, 53%), **7b** (0,12 g, 45%), and **7c** (0,10 g, 36%).

*(1*R*,5*R*,6*R*)-4-Methylidene-6-(2-phenylethyl)-2,7-dioxabicyclo[3.3.0]octan-3,8-dione* (**7a**). Colorless oil. R_f (hexane/AcOEt 1:1) 0.56. $[\alpha]_D^{24} = +76.7$ ($c = 1.46$, CH_2Cl_2). IR (film): 3061, 3027, 2925, 2856, 1789, 1665, 1603, 1497, 1455, 1297, 1221, 1105, 1064, 752, 702. ^{1}H-NMR ($CDCl_3$, 400 MHz): 2.10–2.15 (*m*, CH_2(9)); 2.75–2.92 (*m*, CH_2(10)); 3.54–3.58 (*m*, H–C(5)); 4.41 (*dt*, ^3J(6,5) = 4.1, ^3J(6,9) = 6.6, H–C(6)); 5.05 (*d*, ^3J(1,5) = 8.4, H–C(1)); 5.81 (*d*, ^2J(11a,11b) = 2.2, H_b–C(11)); 6.44 (*d*, ^2J(11b,11a) = 2.2, H_a–C(11)); 7.19–7.34 (*m*, H–C(2′), H–C(3′), H–C(4′), H–C(5′), H–C(6′)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 31.3 (C(10)); 37.8 (C(9)); 44.3 (C(5)); 74.1 (C(1)); 84.0 (C(6)); 126.4 (C(11)); 126.7 (C(4′)); 128.3 (C(2′), C(6′)); 128.8 (C(3′), C(5′)); 134.3 (C(4)); 139.5 (C(1′)); 167.3 (C(3)); 169.6 (C(8)). Anal. calc. for $C_{15}H_{14}O_4$ (258.27): C 69.76, H 5.46; found: C 69.87, H 5.66.

*(1*R*,5*R*,6*R*)-4-Methylidene-6-[2-(4-methylphenyl)ethyl]-2,7-dioxabicyclo[3.3.0]octan-3,8-dione* (**7b**). White solid. R_f (hexane/AcOEt 1:1) 0.57. M.p. 91.1–93.4° (hexane/AcOEt 1:1). $[\alpha]_D^{21} = +68.7$ ($c = 1.15$, CH_2Cl_2). IR (KBr): 3044, 2923, 2862, 1791, 1760, 1663, 1515, 1297, 1240, 1128, 1038, 1019, 979, 818. ^{1}H-NMR ($CDCl_3$, 200 MHz): 2.02–2.21 (*m*, CH_2(9)); 2.33 (*s*, Me); 2.67–2.88 (*m*, CH_2(10)); 3.53–3.60 (*m*, H–C(5)); 4.32–4.45 (*m*, H–C(6)); 5.06 (*d*, ^3J(1,5) = 8.5, H–C(1)); 5.81 (*d*, ^2J(11a,11b) = 2.1, H_b–C(11)); 6.43 (*d*, ^2J(11b,11a) = 2.1, H_a–C(11)); 7.06–7.15 (*m*, H–C(2′), H–C(3′), H–C(5′), H–C(6′)). ^{13}C-NMR ($CDCl_3$, 50 MHz): 21.0 (Me); 30.8 (C(10)); 37.8 (C(9)); 44.2 (C(5)); 74.2 (C(1)); 84.1 (C(6)); 126.4 (C(11)); 127.4 (C(3′), C(5′)); 128.2 (C(2′), C(6′)); 134.3 (C(4)); 136.2 (C(4′)); 136.4

(C(1′)); 167.4 (C(3)); 169.7 (C(8)). Anal. calc. for $C_{16}H_{16}O_4$ (272.30): C 70.57, H 5.92; found: C 70.41, H 5.85.

*(1*R*,5*R*,6*R*)-4-Methylidene-6-{2-[4-(trifluoromethyl)phenyl]ethyl}2,7-dioxabicyclo[3.3.0]octan-3,8-dione* (**7c**). Colorless oil. R_f (hexane/AcOEt 1:1) 0.61. $[\alpha]_D^{26} = +37.5$ ($c = 1.60$, CH_2Cl_2). IR (film): 3118, 2960, 2918, 2868, 1782, 1666, 1617, 1418, 1323, 1194, 1118, 1049, 1019, 846, 594. ^{1}H-NMR ($CDCl_3$, 400 MHz): 2.10–2.19 (*m*, CH_2(9)); 2.80–2.88 (*m*, H_a–C(10)); 2.93–3.00 (*m*, H_b–C(10)); 3.56–3.60 (*m*, H–C(5)); 4.35–4.42 (*m*, H–C(6)); 5.08 (*d*, ^3J(1,5) = 6.4, H–C(1)); 5.85 (*d*, ^2J(11a,11b) = 2.4, H_b–C(11)); 6.46 (*d*, ^2J(11b,11a) = 2.4, H_a–C(11)); 7.33 (*d*, ^3J(2′,3′) = 8.0, ^3J(6′,5′) = 8.0, H–C(2′), H–C(6′)); 7.58 (*d*, ^3J(3′,2′) = 8.0, ^3J(5′,6′) = 8.0, H–C(3′), H–C(5′)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 31.3 (C(10)); 37.8 (C(9)); 44.4 (C(5)); 74.1 (C(1)); 83.8 (C(6)); 125.8 (*q*, ^3J(C,F) = 3.7, C(3′), C(5′)); 126.5 (C(11)); 128.7 (C(2′), C(6′)); 129.1 (*q*, ^2J(C,F) = 32.7, C(4′)); 134.2 (C(4)); 143.7 (C(1′)); 167.2 (C(3)); 169.5 (C(8)). Anal. calc. for $C_{16}H_{13}F_3O_4$ (326.27): C 58.90, H 4.02; found: C 58.56, H 4.33.

Biological Assay. Four sterilized *Blank* paper disks (6 mm) were dipped into the solns. of the bis-γ-lactones **6a**–**6c**, **7a**–**7c**, and avenaciolide (1000 ppm) in CH_2Cl_2. After 5 min, the disks were allowed to dry in a desiccator at reduced pressure. The same procedure was employed in the preparation of the positive control (the commercial fungicide *Folicur*). The negative check treatment was prepared with solvent only. Each disk was placed in the center of a *Petri* dish containing *Colletotrichum gloeosporioides* spores (isolated from infected tissues of papaya) over the potato dextrose agar medium (*DIFCO*). The distances from the center of the disks to the edge of the inhibition zone, from where a dense and filamentous growth of the fungus could be observed with the aid of a stereoscopic microscope, were measured after 48 h at 25°. Compounds **6a**–**6c** were inactive. The *Petri* dishes containing the bis-γ-lactones **7a**–**7c** and avenaciolide showed transparent hales of 1.5, 1.5, 1.8, and 1.7 cm diameter, resp., inside a larger hale containing some micelial growth. Tebuconazole (*Folicur*) presented an inhibition hale of 3.8 cm.

REFERENCES

[1] D. Brookes, B. K. Tidd, W. B. Turner, *J. Chem. Soc.* **1963**, *68*, 5385.
[2] R. C. Anderson, B. Fraser-Reid, *J. Am. Chem. Soc.* **1975**, *97*, 3870.
[3] D. L. Hughes, *Acta Crystallogr., Sect. B* **1978**, *34*, 3674.
[4] J. Meyer, P. M. Vignais, *Biochim. Biophys. Acta* **1973**, *325*, 375.
[5] D. Litwinska, A. Szczesna-Kaczmarek, J. Popinigis, *Int. J. Biochem.* **1984**, *16*, 943.
[6] V. S. Martín, C. M. Rodríguez, T. Martín, *Org. Prep. Proced. Int.* **1998**, *30*, 291.
[7] S. Braukmüller, R. Brückner, *Eur. J. Org. Chem.* **2006**, 2110.
[8] A. S. Magaton, M. M. M. Rubinger, F. C. Macedo-Júnior, L. Zambolim, *J. Braz. Chem. Soc.* **2007**, *18*, 284.
[9] A. K. Picman, *Biochem. Syst. Ecol.* **1986**, *14*, 255.
[10] S. Czernecki, C. Georgoulouis, C. L. Stevens, K. Vijayakumaran, *Tetrahedron Lett.* **1985**, *26*, 1699.
[11] A. Rosenthal, L. Nguyen, *J. Org. Chem.* **1969**, *34*, 1029.
[12] W. L. Parker, F. Johnson, *J. Org. Chem.* **1973**, *38*, 2489.
[13] D. Brookes, S. Sternhell, B. K. Tidd, W. B. Turner, *Aust. J. Chem.* **1965**, *18*, 373.
[14] K. Lee, Y. Wu, I. H. Hall, *J. Med. Chem.* **1977**, *20*, 911.
[15] M. Miyazawa, H. Shimabayashi, S. Hayashi, S. Hashimoto, S. Nakamura, H. Kosaka, H. Kameoka, *J. Agric. Food. Chem.* **2000**, *48*, 5406.
[16] A. J. Scott, M. Knott, *Biometrics* **1974**, *30*, 507.

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