Pergamon

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Tetrahedron: *Asymmetry* 14 (2003) 3755–3760

TETRAHEDRON: *ASYMMETRY*

Studies on biotransformation of (±)-1-(4′-chlorophenyl)-2-phenylethanol

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Received 29 July 2003; accepted 27 August 2003

Abstract—The metabolism of (±)-1-(4′-chlorophenyl)-2-phenylethanol **1** by the phytopathogenic fungi *Colletotrichum gloeosporioides* and *Botrytis cinerea* has been studied. Regioselective monooxygenase-catalyzed hydroxylation of C–H bonds yielded the corresponding diols, the ratios of which showed a clear preference for the C–H benzylic bond. *m*-Hydroxylation in the substituted aromatic ring, a rarely described phenomenon, has been observed and with good enantioselectivity. The chemistry involved in determining the configuration of the biotransformation products is also described.

1. Introduction

Over the past few years, our research programme has been directed towards the rational design of fungicides for use against fungal infections of commercial crops. Since phytoalexins are known to exhibit significant antifungal activity,[1] we have undertaken a screening of analogous compounds[2] in order to find substrates with similar antifungal properties.

There are, however, many reports on the degradation of plant anti-microbial compounds by phytopathogenic fungi via a variety of mechanisms; for example, isolates of *Botrytis cinerea* have been shown to detoxify the grapevine phytoalexin resveratrol.[3] In many cases the degradation products are actually less toxic to fungal growth than the parent compounds.[4] Some fungi have even been reported to detoxify more than one plant compound.

Our investigations have thus focused on the metabolism of diverse compounds that are analogous to the phytoalexins produced by the agriculturally important pathogens *Botrytis cinerea* and *Colletotrichum gloeosporioides*. These two species are highly destructive plant-pathogenic fungi, responsible for diseases such as grey mould in grapes and anthracnose in strawberry fruits and plants.[5,6] Based on previous results, from which the presence of a hydroxyl group was determined to be fundamental for the expression of the antifungal activity of the compounds studied,[7,8] we have thus chosen to study the resveratrol analogue (±)-1-(4′-chlorophenyl)-2-phenylethanol **1**, which contains a secondary hydroxyl group in its structure.

2. Results and discussion

One of our main objectives was to elucidate the metabolism of (±)-1-(4′-chlorophenyl)-2-phenylethanol **1** by *C. gloeosporioides* and *B. cinerea* (Scheme 1) and to characterize and, where possible, to assign the stereochemistry to the metabolites.

Compound **1**, obtained by means of chemical reduction of commercial benzyl 4′-chlorophenyl ketone **2**, was incubated for nine days on shaken cultures of either *C. gloeosporioides* or *B. cinerea* at a concentration of 150 ppm on a Czapek-Dox medium and then extracted with ethyl acetate. As a control, cultures of only the fungi or a medium containing only compound **1** were also incubated under similar conditions and extracted. These control extracts were compared with those from the biotransformation experiments by means of TLC.

In addition to the starting material, the biotransformation of (±)-1-(4′-chlorophenyl)-2-phenylethanol **1** by *C.*

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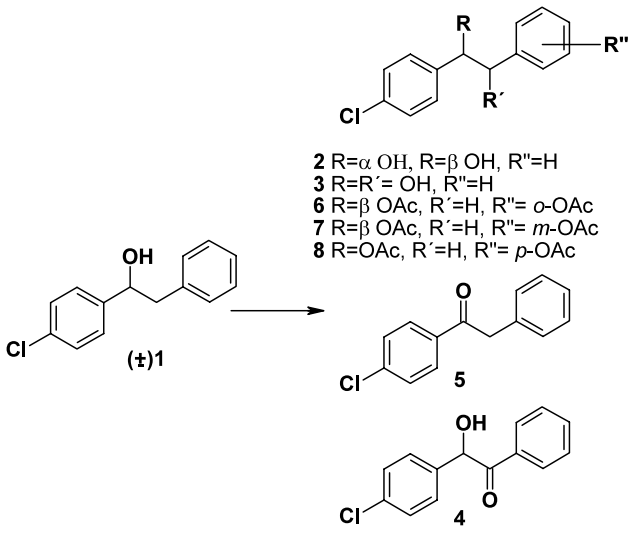
doi:10.1016/j.tetasy.2003.08.026



Scheme 1. Biotransformation of **1** by *C. gloeosporoides*.

gloeosporioides afforded four compounds: (1*RS*,2*SR*)-1-(4′-chlorophenyl)-2-phenylethane-1,2-diol **2**, (1*S*,2*S*)-1-(4′-chlorophenyl)-2-phenylethane-1,2-diol **3**, (*R*)-2-hydroxy-2-(4′-chlorophenyl)acetophenone **4** and benzyl 4′-chlorophenyl ketone **5**. The acetylation of the polar fraction with acetic anhydride–pyridine led to the isolation of an additional three compounds: (1*R*)-2-(2′-acetoxyphenyl)-1-(4″-chlorophenyl)ethyl acetate **6**, (1*R*)-2-(3′-acetoxyphenyl)-1-(4″-chlorophenyl)ethyl acetate **7** and (±)-2-(4′-acetoxyphenyl)-1-(4″-chlorophenyl)ethyl acetate **8**.

The recovered starting material showed $[\alpha]_D^{20} = -0.2$ ($CHCl_3$, $c = 1.8$): with 14.1% ee, indicating that the fungus exhibited a slight preference for the enantiomer (1*R*)-(+)-1-(4′-chlorophenyl)-2-phenylethanol (*R*)-**1** in the biotransformation. The absolute configuration of the residual alcohol was proposed to be *S* after its specific rotation sign was compared with that described for (1*R*)-1-(4-fluorophenyl)-2-phenylethan-1-ol, which was obtained by means of a chemical reaction via boronic esters.[9] In addition, alcohol (2*S*)-(−)-**1** was obtained with 99% ee and 98% yield, by means of treatment of benzyl 4′-chlorophenyl ketone **2** with BH_3–THF and (*S*)-methyl oxazaboralidine.[10]

Diols **2** and **3** were the two major biotransformation products. Comparison of their spectra with that of starting material **1** indicated that these compounds were two diasteromers of 1-(4′-chlorophenyl)-2-phenylethane-1,2-diol $[\alpha]_D^{20} = 0$ ($CHCl_3$, $c = 0.7$): for **2** and $[\alpha]_D^{20} = -100.4$ ($CHCl_3$, $c = 4.2$): with 91.3% ee for **3**. The hydroxylation on C-2 of **1** indicates that this biotransformation is most likely due to the action of a monooxygenase.

The absolute configuration of compound **3** was assigned as (1*S*,2*S*) on the basis of two considerations. First, this assignment is in agreement with both the preferred configurations of diols produced using α- and β-forms of AD-mix and the model developed by Sharpless for predicting the enantiofacial selectivity of the dihydroxylation process for different alkene types.[11,12] Second, comparison of the specific rotations of the obtained diols with those described in the literature for (1*R*,2*R*)-1-(4′-chlorophenyl)-2-phenylethane-1,2-diol[13] and (1*R*,2*R*) and (1*S*,2*S*)-1,2-diphenyl-1,2-ethane-1,2-diol supported this assignment.[14]

Asymmetric dihydroxylation of (*E*)-1-(4′-chlorophenyl)-2-phenylethene **10**, prepared from **1** by acidic treatment using α and β AD-mix (H_2O:*t*-BuOH, 20°C) led to (1*S*,2*S*)-**3** and (1*R*,2*R*)-**3**, respectively. The enantiomeric excess values observed for these diols were found to be over 99%.

Further metabolism of diol **2** led to the ketoalcohol **4** (ν_{max} 1679 cm^{-1}, δ_C 198.6 ppm; ν_{max} 3418 cm^{-1}, δ_C 75.4 ppm), which was found to be present with an excess of the (*R*) enantiomer (7.5% ee). The absolute configuration was tentatively assigned as (*R*) after comparison of its specific rotation sign with those of commercial (*R*)- and (*S*)-benzoin. This transformation seems to be due to the action of a dehydrogenase enzyme.

Isolation of ketone **5** from the fermentation broth confirmed the presence of a dehydrogenase. The spectroscopic data of **5** were identical to those of the commercial product benzyl 4′-chlorophenyl ketone **5**.

It is interesting to note that while the hydroxylation of aromatics in the *o*- and *p*-positions to existing substituents is frequently achieved by means of monooxygenases, *m*-hydroxylation is rarely observed.[15]

During the metabolism of **1**, however, we observed the hydroxylation in all three positions. Thus the respective spectroscopic data of compounds **6**, **7** and **8**, which were isolated from the fermentation broth after acetylation, not only pointed to hydroxylation in the *o*-, *m*- and *p*-positions of the aromatic ring without a chloro substituent, but also confirmed that these compounds were in fact 2-(2′-acetoxyphenyl)-1-(4″-chlorophenyl)-ethyl acetate **6**, 2-(3′-acetoxyphenyl)-1-(4″-chlorophenyl)ethyl acetate **7** and 2-(4′-acetoxyphenyl)-1-(4″-chlorophenyl)ethyl acetate **8**. Compounds **6** and **7** showed $[\alpha]_D^{20} = -3.6$ ($CHCl_3$, $c = 0.8$): 92.7% ee, and $[\alpha]_D^{20} = -4.0$ ($CHCl_3$, $c = 0.5$): 94.8% ee, respectively, indicating that monooxygenase exhibited a high preference for the (*R*) enantiomer. Compound **8** was found to be racemic. The absolute configurations of alcohols **6** and **7** were both assigned as (*R*) after their specific rotation signs were compared with those obtained for (1*S*)-(−)-1-(4-chlorophenyl)-2-phenylethyl acetate **9**. Compound **9** was obtained after acetylation of alcohol (1*S*)-(−)-**1**.

In contrast, no biotransformation products were obtained during the feeding experiment of *B. cinerea* with (±)-1-(4′-chlorophenyl)-2-phenylethanol **1** at 150 ppm. Mycelial growth was not observed during the biotransformation and study of the fermentation broth only led to the isolation of fatty acids, indicating that *B. cinerea* was unable to detoxify this compound. One

reason for this may be that the concentration of the starting material was too high for the fungus, and thus inhibited fungal growth.

To confirm this, (±)-1-(4′-chlorophenyl)-2-phenylethanol **1** was incubated at a lower concentration (100 ppm) under the same conditions on shaken cultures of *B. cinerea*. In this case, in addition to the starting material, the biotransformation of (±)-1-(4′-chlorophenyl)-2-phenylethanol **1** afforded benzyl 4′-chlorophenyl ketone **4**. The remaining alcohol **1** was found to be of low enantiopurity (6% ee) and of the (1*R*) configuration.

3. Conclusions

The biotransformation of (±)-1-(4-chlorophenyl)-2-phenylethanol **1** by *C. gloeosporioides* led to hydroxylation and further oxidation at C-1 and C-2, as well as to hydroxylation of the aromatic ring. The lack of hydroxylation of the 4-chloro aromatic ring indicated the regioselectivity of the monooxygenase.

In incorporation experiments of (±)-1-(4-chlorophenyl)-2-phenylethanol **1** at a concentration of 150 ppm, *B. cinerea* was not able to detoxify this compound. When the biotransformation was carried out at a lower concentration, a ketone derivative was obtained as the only product.

In conclusion, fungal biodegradation of (±)-1-(4-chlorophenyl)-2-phenylethanol **1** using *C. gloeosporioides* and *B. cinerea* has generally been found to proceed via a monooxygenase-catalyzed hydroxylation of C–H bonds to yield compounds **2**, **3**, **6**, **7** and **8**. The compound ratios indicate a clear preference for the C–H benzylic bond. *m*-Hydroxylation in the substituted aromatic ring, which is rarely described, has been observed with high enantioselectivity (94.8% ee). In addition, dehydrogenase-catalyzed oxidation occurred at secondary benzylic groups to yield ketones **4** and **5**.

4. Experimental

4.1. General experimental procedures

Optical rotations were determined with a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Mattson Genesis spectrophotometer, series FTIR. ^{1}H and ^{13}C NMR measurements were obtained on Varian Gemini 200 and Varian Unity 400 NMR spectrometers with $SiMe_4$ as the internal reference. Mass spectra were recorded on a GC–MS Thermoquest spectrometer, model Voyager, and a VG Autospec-Q spectrometer. HPLC was performed with a Hitachi/Merck L-6270 apparatus equipped with a UV–vis detector (L 4250) and a differential refractometer detector (RI-71). TLC was performed on Merck Kiesegel 60 F_{254}, 0.2 mm thick. Silica gel (Merck) was used for column chromatography. Purification by means of HPLC was accomplished using a silica gel column (Hibar 60, 7 m, 1 cm wide, 25 cm long). Chemicals were products of Fluka or Aldrich. All solvents used were freshly distilled. Enantiomeric excesses were determined by means of HPLC analyses on a chiral column (Chiralcel OD, Daicel, Japan).

4.2. Biotransformation by *Colletotrichum gloeosporioides*

The culture of *Colletotrichum gloeosporioides* employed in this work, 20122-CECT, was obtained from the 'Centro Español de Cultivos Tipo' (CECT), Facultad de Biología, Universidad de Valencia, Spain, where a culture of this strain is deposited.

C. gloeosporioides was grown on an agar-malta-peptone medium in a Petri dish. Ten-day-old mycelium was used to inoculate 28 (500 ml) flasks, each containing 250 ml of Czapek-Dox medium: glucose (50 g), yeast extract (1 g), KH_2PO_4 (5 g), $NaNO_3$ (2 g), $MgSO_4$ (0.5 g) and $FeSO_4$ (10 mg), per litre of distilled water. The pH was adjusted to 7.0 with aqueous NaOH. For 2 days, flasks were incubated at 25°C and stirred at 180 rpm; the mycelium was then filtered and transferred into 26 (500 ml) flasks containing 250 ml of Czapek-Dox medium (with only 25 g of glucose) and the substrate ((±)-1-(4′-chlorophenyl)-2-phenylethanol **1**, 150 ppm per flask). The remaining two flasks were used as the control. 9 days after inoculation, the mycelium was filtered and the fermentation broth was extracted three times with ethyl acetate. The extract was dried over anhydrous sodium sulphate and the solvent was then evaporated. Fractionation of the extract (870 mg) was carried out by means of column chromatography on silica gel (SiCC), eluting with petroleum ether: ethyl acetate mixtures containing increasing percentages of ethyl acetate to give 5 fractions (A–E). Final purification of fraction A (40 mg, petroleum ether) was carried out by means of semi-preparative HPLC (hexane:ethyl acetate 97:3; 2.5 mL min^{-1}) to afford benzyl 4′-chlorophenyl ketone **5** (5 mg). Compounds **1** and **4** were obtained after further purification of fraction B (409 mg, petroleum ether: ethyl acetate 85:15) with SiCC under conditions similar to those described above. Final purification was carried out by means of semi-preparative HPLC (hexane:ethyl acetate 90:10; 3.2 mL min^{-1}) to afford recovered 1-(4′-chlorophenyl)-2-phenylethanol **1** (211 mg) and (2*R*)-2-(4′-chlorophenyl)-2-hydroxy-1-phenylethanone **4** (3 mg, 7.5% ee). Diols **2** (*RS*,*SR*)-1-(4′-chlorophenyl)-2-phenylethane-1,2-diol (98 mg) and **3** (1*S*,2*S*)-1-(4′-chlorophenyl)-2-phenylethane-1,2-diol (96 mg, 91.3% ee), eluted in fraction C (280 mg, petroleum ether: ethyl acetate 70:30), were obtained via purification on semi-preparative HPLC (hexane:ethyl acetate 70:30; 3.1 mL min^{-1}).

Acetylation of the polar fractions (D–E, 51 mg) followed by final purification with semi-preparative HPLC (hexane:ethyl acetate 88:12; 3.3 mL min^{-1}) led to the isolation of the following compounds: (1*R*)-2-(2′-acetoxyphenyl)-1-(4″-chlorophenyl)ethyl acetate **6** (10 mg, 92.7% ee), (1*R*)-2-(3′-acetoxyphenyl)-1-(4″-chlorophenyl)ethyl acetate **7** (16 mg, 94.8% ee) and

(±)-2-(4′-acetoxyphenyl)-1-(4″-chlorophenyl)ethyl acetate **8** (7 mg).

4.2.1. (1*S*)-1-(4′-Chlorophenyl)-2-phenylethanol 1.[16] White solid; mp 46–48°C; $[\alpha]_D^{20}=-0.2$ ($CHCl_3$, $c=1.8$): 14.1% ee. The enantiomeric excess was measured by means of chiral HPLC with *n*-hexane/*i*-PrOH 97:3 as eluent, flow rate 0.8 mL/min, t_R/min = 20.3 ((*S*)-**1**), 29.0 ((*R*)-**1**).

4.2.2. (1*RS*,2*SR*)-1-(4′-Chlorophenyl)-2-phenylethane-1,2-diol 2. White solid; mp 119–121°C; $[\alpha]_D^{20}=0$ ($CHCl_3$, $c=0.7$): IR ν_{max} (film): 3358, 3296, 2901, 1028 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.29 (1H, s, OH), 2.34 (1H, s, OH), 4.81 (2H, bs, H-1, H-2), 7.12 (2H, d, $J=8.4$ Hz, H-2′, H-6′), 7.17–7.21 (2H, m, H-6″, H-2″), 7.24 (2H, d, $J=8.5$ Hz, H-3′, H-5′), 7.27–7.31 (3H, m, H-3″, H-5″, H-4″); ^{13}C NMR (100 MHz, $CDCl_3$): δ 77.3 (d, C-1), 77.9 (d, C-2), 126.9 (d, C-2″, C-6″), 128.1 (d, C-2′, C-6′, C-4″), 128.2 (d, C-3″, C-5″), 128.3 (d, C-3′, C-5′), 133.6 (s, C-4′), 138.0 (s, C-1′), 139.2 (s, C-1″); EIMS *m*/*z*: 232 (M^+−18+2, 2), 230 (M^+−18, 8), 214 (M^+−18−18+2, 28), 212 (M^+−18−18, 5), M^+−36; 179 (40), 178 (43), 139 (77), 111 (48), 105 (86), 77 (100); HREIMS *m*/*z* 248.0600 [M^+] (calcd. for $C_{14}H_{13}O_2Cl$, 248.0626).

4.2.3. (1*S*,2*S*)-1-(4′-Chlorophenyl)-2-phenylethane-1,2-diol 3. White solid; mp 100–102°C; $[\alpha]_D^{20}=-100.4$ ($CHCl_3$, c = 4.2): 91.3% ee; IR ν_{max} (film): 3400, 3061, 3031, 2930, 1493, 1091, 1017 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.13 (1H, s, OH), 3.27 (1H, s, OH), 4.53–4.61 (2H, m, H-1, H-2), 6.94–6.99 (2H, m, H-2′, H-6′), 7.02–7.08 (2H, m, H-6″, H-2″), 7.13–7.18 (2H, m, H-3′, H-5′), 7.20–7.25 (3H, m, H-3″, H-5″, H-4″); ^{13}C NMR (100 MHz, $CDCl_3$): δ 78.4 (d, C-1), 79.1 (d, C-2), 126.9 (d, C-2″, C-6″), 128.0 (d, C-4″), 128.08 (d, C-2′, C-6′), 128.1 (d, C-3″, C-5″), 128.2 (d, C-3′, C-5′), 133.4 (s, C-4′), 138.1 (s, C-1′), 139.3 (s, C-1″); EIMS *m*/*z*: 214 (M^+−36, 58), 179 (70), 178 (83), 139 (75), 111 (58), 105 (94), 77 (100); HREIMS *m*/*z* 248.0619 [M^+] (calcd. for $C_{14}H_{13}O_2Cl$, 248.0604). The enantiomeric excess was determined to be diacetate (see general procedure), measured by means of chiral HPLC with *n*-hexane/*i*-PrOH 99.7:0.3 as eluent, flow rate 0.8 mL/min, t_R/min = 34.0 ((*S*,*S*)-**3Ac**), 49.2 ((*R*,*R*)-**3Ac**).

4.2.4. (2*R*)-2-Hydroxy-2-(4′-chlorophenyl) acetophenone 4. White solid; mp 88–90°C; $[\alpha]_D^{20}=-0.8$ ($CHCl_3$, $c=0.3$): 7.5% ee; IR ν_{max} (film): 3418, 3063, 2917, 2858, 1679, 1595, 1087 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.54 (1H, s, OH), 5.92 (1H, s, H-2), 7.26 (2H, d, $J=8.3$ Hz, H-2′, H-6′), 7.29 (2H, d, $J=8.1$ Hz, H-3′, H-5′), 7.40 (2H, ddd, $J=7.7$, 7.7, 3.3 Hz, H-3″, H-5″), 7.54 (1H, ddd, $J=7.4$, 7.4, 2.5 Hz, H-4″), 7.88 (2H, bdd, $J=8.0$, 3.4 Hz, H-2″, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$): δ 75.4 (d, C-2), 128.8 (d, C-3″, C-5″), 129.1 (d, C-2″, C-6″, C-3′, C-5′), 129.3 (d, C-2′, C-6′), 133.2 (s, C-4′), 134.1 (d, C-4″), 134.5 (s, C-1′), 137.5 (s, C-1″), 198.6 (s, C-1); EIMS *m*/*z*: 139(28), 141 (45), 111 (38), 105 (100), 77 (60); HREIMS *m*/*z* 246.0473 [M^+] (calcd. for $C_{14}H_{11}O_2Cl$, 246.0506). The enantiomeric excess was measured by means of chiral HPLC with *n*-hexane/*i*-PrOH 90:10 as eluent, flow rate 1.0 mL/min, t_R/min = 7.4 ((*R*)-**4**), 10.12 ((*S*)-**4**).

4.2.5. Benzyl (4′-chlorophenyl) ketone 5. White solid; mp 102–103°C; IR ν_{max} (film): 3093, 3027, 2898, 1687, 1589, 1090, 1071 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.24 (2H, s, H-2), 7.25 (3H, ddd, $J=7.1$, 7.1, 2.9 Hz, H-2″, H-6″, H-4″), 7.33 (2H, ddd, $J=7.2$, 7.1, 2.9 Hz, H-3″, H-5″), 7.41 (2H, d, $J=8.7$ Hz, H-3′, H-5′), 7.93 (2H, d, $J=8.8$ Hz, H-2′, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 45.5 (t, C-2), 126.9 (d, C-4″), 128.6 (d, C-3′, C-5′), 128.8 (d, C-3″, C-5″), 129.3 (d, C-2″, C-6″), 129.9 (d, C-2′, C-6′), 134.0 (s, C-1′), 134.6 (s, C-1″), 139.5 (s, C-4′), 196.2 (s, C-1); EIMS *m*/*z*: 230 (M^+, 5), 165 (15); 141 (95), 139 (100), 113 (50), 111 (90), 91 (93), 75 (80).

4.2.6. (1*R*)-2-(2′-Acetoxyphenyl)-1-(4″-chlorophenyl)-ethyl acetate 6. Colourless oil; $[\alpha]_D^{20}=-3.6$ ($CHCl_3$, $c=0.8$): 92.7% ee; IR ν_{max} (film): 3023, 2925, 2853, 1739, 1235 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.03 (3H, s, CH_3COO), 2.35 (3H, s, CH_3COO), 2.85 (1H, dd, $J=13.6$, 7.4 Hz, H-2a), 3.20 (1H, dd, $J=13.6$, 6.8 Hz, H-2b), 5.87 (1H, dd, $J=7.4$, 6.8 Hz, H-1), 7.01–7.07 (3H, m, H-3′, H-5′, H-4′), 7.14 (2H, d, $J=8.5$ Hz, H-2″, H-6″), 7.20–7.23 (1H, m, H-6′), 7.25 (2H, d, $J=8.4$ Hz, H-3″, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.0 (c, $\underline{C}H_3COO$), 21.1 (c, $\underline{C}H_3COO$), 37.5 (t, C-2), 74.7 (d, C-1), 122.4 (d, C-3′), 125.8 (d, C-5′), 127.9 (d, C-4′), 128.1 (d, C-6′), 128.5 (d, C-2″, C-6″), 131.6 (d, C-3″, C-5″), 133.8 (s, C-1′, C-4″), 138.1 (s, C-1″), 149.4 (s, C-2′), 169.5 (s, $CH_3\underline{C}OO$), 170.0 (s, $CH_3\underline{C}OO$); EIMS *m*/*z*: 274 (M^+−60+2, 9), 272 (M^+−60, 14), 232 (38), 230 (93), 195 (18), 165 (51), 141 (26), 107, (100), 77 (20); HREIMS *m*/*z* 332.0811 [M^+] (calcd. for $C_{18}H_{17}O_4Cl$, 332.0837). The enantiomeric excess was measured by means of chiral HPLC with *n*-hexane/*i*-PrOH 90:10 as eluent, flow rate 0.8 mL/min, t_R/min = 10.6 ((*R*)-**6**), 16.5 ((*S*)-**6**).

4.2.7. (1*R*)-2-(3′-Acetoxyphenyl)-1-(4″-chlorophenyl)-ethyl acetate 7. Colourless oil; $[\alpha]_D^{20}=-4.0$ ($CHCl_3$, $c=0.5$): 94.8% ee; IR ν_{max} (film): 3044, 2930, 2860, 1763, 1209 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.01 (3H, s, CH_3COO on C-3′), 2.27 (3H, s, CH_3COO on C-1), 3.0 (1H, dd, $J=13.7$, 6.3 Hz, H-2a), 3.15 (1H, dd, $J=13.8$, 7.7 Hz, H-2b), 5.87 (1H, dd, $J=7.5$, 6.5 Hz, H-1), 6.83 (1H, bs, H-4′), 6.92 (2H, bd, $J=7.9$ Hz, H-2′, H-6′), 7.17 (2H, d, $J=8.5$ Hz, H-2″, H-6″), 7.22 (1H, dd, $J=7.7$, 7.7 Hz, H-5′), 7.27 (2H, d, $J=8.4$ Hz, H-3″, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.1 (c, $\underline{C}H_3COO$), 21.2 (c, $\underline{C}H_3COO$), 42.6 (t, C-2), 75.6 (d, C-1), 119.8 (d, C-4′), 122.6 (d, C-2′), 126.9 (d, C-6′), 127.8 (d, C-2″, C-6″), 128.5 (d, C-3″, C-5″), 129.2 (d, C-5′), 133.7 (s, C-4″), 138.10 (s, C-1″), 138.1 (s, C-1′), 150.4 (s, C-3′), 169.3 (s, $CH_3\underline{C}OO$), 169.9 (s, $CH_3\underline{C}OO$); EIMS *m*/*z*: 274 (M^+−60+2, 17), 272 (M^+−60, 46), 232 (48), 230 (65), 195 (26), 165 (77), 150 (100), 141 (95), 107 (88), 77 (52); HREIMS *m*/*z* 332.0814 [M^+] (calcd. for $C_{18}H_{17}O_4Cl$, 332.0837). The enantiomeric excess was measured by means of chiral HPLC with *n*-hexane/*i*-PrOH 90:10 as eluent, flow rate 0.8 mL/min, t_R/min = 10.12 ((*R*)-**7**), 15.32 ((*S*)-**7**).

4.2.8. (±)-2-(4′-Acetoxyphenyl)-1-(4″-chlorophenyl)ethyl acetate 8. Colourless oil; $[\alpha]_D^{20}=0$ ($CHCl_3$, $c=0.4$): IR ν_{max} (film): 3037, 2928, 2854, 1739, 1234, 1196 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.02 (3H, s, CH_3COO), 2.27 (3H, s, CH_3COO), 2.99 (1H, dd, $J=13.8$, 6.5 Hz, H-2a), 3.16 (1H, dd, $J=13.8$, 7.5 Hz, H-2b), 5.86 (1H, dd, $J=6.9$, 6.9 Hz, H-1), 6.96 (2H, d, $J=8.7$ Hz, H-3′, H-5′), 7.06 (2H, d, $J=8.6$ Hz, H-2′, H-6′), 7.17 (2H, d, $J=8.5$ Hz, H-2″, H-6″), 7.27 (2H, d, $J=8.5$ Hz, H-3″, H-5″); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.2 (c, 2CH_3COO), 42.2 (t, C-2), 75.8 (d, C-1), 121.3 (d, C-3′, C-5′), 127.9 (d, C-2′, C-6′), 128.5 (d, C-2″, C-6″), 130.4 (d, C-3″, C-5″), 133.7 (s, C-4″), 134.0 (s, C-1′), 138.2 (s, C-1″), 149.3 (s, C-4′), 169.4 (s, CH_3C=OO), 169.9 (s, CH_3C=OO); EIMS m/z: 274 (M^+−60+2, 8), 272 (M^+−60, 20), 232 (36), 230 (100), 195 (12), 165 (48), 150 (34), 141 (42), 107 (94), 77 (30); HREIMS m/z 332.0800 [M^+] (calcd. for $C_{18}H_{17}O_4Cl$, 332.0815).

4.3. Biotransformation by *B. cinerea*

The culture of *Botrytis cinerea* employed in this work, *B. cinerea* 2100, was obtained from the 'Centro Español de Cultivos Tipo' (CECT), Facultad de Biología, Universidad de Valencia, Spain, where a culture of this strain is deposited.

Biotransformation of (±)-1-(4′-chlorophenyl)-2-phenylethanol **1** by *B. cinerea* was carried out as described above for the *Colletotrichum* experiment (at a concentration of 150 ppm), but in this case, the fungus was inoculated by conidia. No biotransformation products were obtained.

However, at a lower substrate concentration (100 ppm per flask), in addition to compounds characteristic of *B. cinerea*, the following biotransformation products were also isolated from the extract (847 mg): recovered 1-(4′-chlorophenyl)-2-phenylethanol **1** (410 mg, $[\alpha]_D^{20}=+3.1$ ($CHCl_3$, $c=0.5$): 6% ee and benzyl 4′-chlorophenyl ketone **2** (44 mg).

4.4. Chemical transformations

4.4.1. (±)-1-(4′-Chlorophenyl)-2-phenylethan-1-ol 1. To 1000 mg of benzyl 4-chlorophenyl ketone dissolved in a mixture of dichlomethane:methanol 1:1, 400 mg of $NaBH_4$ were added. The reaction mixture was stirred at room temperature for 1 h. Distillation under reduced pressure to eliminate the solvent led to a crude mixture that was neutralised with aqueous HCl 10% and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and the solvent was eliminated by means of distillation under reduced pressure to yield 925.6 mg (91%) of **1**.[16]

4.4.2. (1*S*)-(−)-1-(4′-Chlorophenyl)-2-phenylethan-1-ol (−)-1. Under an argon atmosphere, an oven-dried Schlenk tube was charged with (*S*)-methyl oxazaboralidine (0.84 mmol, 0.84 ml of 1 M solution in toluene). The solvent was removed under high vacuum (0.1 mbar) at room temperature and tetrahydrofuran (THF, 11 ml) was added. After the solution was cooled to 0°C, it was treated with a borane–THF complex (1.0 M, 1.0 mL) and stirred at room temperature for 2 h. This reagent and a solution of benzyl 4′-chlorophenyl ketone **5** (155 mg, 0.671 mmol) in THF (10 mL) were then added simultaneously from two syringes into an oven dried, round-bottomed flask at a temperature of 30°C over a period of 1 h. After the reaction mixture was stirred for an additional 1 h, water (20 mL) was added, the organic solvent was removed by means of rotary evaporation and the remaining aqueous phase was extracted with diethyl ether. The combined organic layers were then dried over magnesium sulphate. Removal of the solvent by means of rotary evaporation and subsequent filtration through a short silica gel column afforded 150 mg (98%) of the (*S*) alcohol, $[\alpha]_D^{20}=-23.0$ ($CHCl_3$, $c=2.9$): 99% ee.

4.4.3. (*E*)-1-(4′-Chlorophenyl)-2-phenylethene 10. After dissolution in 1,4-dioxane (15 ml), 3.01 g (12.95 mmol) of (±)-1-(4′-chlorophenyl)-2-phenylethanol **1** were placed in a 500 ml round-bottom flask and 5 ml of H_2SO_4:H_2O 2:1 was added slowly to the reaction mixture. The solution was then stirred at room temperature for 10 min. After this period, it was neutralised with sodium carbonate and extracted with ethyl acetate. The residue was purified by means of chromatography with hexane as eluent to give the compound (*E*)-1-(4′-chlorophenyl)-2-phenylethene **10** (2.53 g, 91%). The ^1H NMR spectrum of this product was in agreement with that found in the literature.[17,18]

4.4.4. Sharpless dihydroxylation

4.4.4.1. (1*S*,2*S*)-1-(4′-Chlorophenyl)-2-phenylethane-1,2-diol (1*S*,2*S*)-3. Distilled water (1.2 ml) and *tert*-butanol (1.2 ml) were added to AD mix α (0.681 g) in a 10 ml round-bottom flask and the resulting suspension was stirred at 0°C. Stirring was maintained during addition of the substrate (*E*)-1-(4′-chlorophenyl)-2-phenylethene **10** (0.098 g, 0.46 mmol) in *tert*-butanol:H_2O 1:1 (1 ml). Then, methanesulphonamide (0.044 g, 0.46 mmol) was added. The reaction mixture was stirred vigorously in the dark at room temperature. After 50 h, anhydrous sodium sulphite (0.731 g, 5.8 mmol) was added and stirring continued for 1 h. After extraction with ethyl acetate (3×20 ml), purification on silica gel afforded the compound (1*S*,2*S*)-1-(4′-chlorophenyl)-2-phenylethane-1,2-diol (1*S*,2*S*)-**3** (0.085 g, 75%), which showed $[\alpha]_D^{20}=-114.2$ ($CHCl_3$, $c=1.9$). This product exhibited >99% ee upon chiral HPLC analysis.

4.4.4.2. (1*R*,2*R*)-1-(4′-Chlorophenyl)-2-phenylethane-1,2-diol (1*R*,2*R*)-3. AD mix β (0.890 g) was added to distilled water (1.5 ml) and *tert*-butanol (1.5 ml). The mixture was stirred at 0°C. Stirring was maintained during addition of the substrate (*E*)-1-(4′-chlorophenyl)-2-phenylethene **10** (0.116 g, 0.54 mmol) in *tert*-butanol:H_2O 1:1 (1.2 ml). Then, methanesulphonamide (0.057 g, 0.54 mmol) was added. The reaction mixture was stirred vigorously in the dark at room temperature. After 24 h, anhydrous sodium sulfite (0.956 g, 7.6 mmol) was added and stirring continued for 1 h. After extraction with ethyl acetate

(3×20 ml), purification on silica gel afforded the compound (1*R*,2*R*)-1-(4′-chlorophenyl)-2-phenylethane-1,2-diol (1*R*,2*R*)-**3** (0.101 g, 75%), which showed $[\alpha]_D^{20}=+105$ ($CHCl_3$, $c=2.8$). This compound exhibited >99% ee upon chiral HPLC analysis.

4.4.5. General acetylation procedure. Starting materials were dissolved in dry pyridine and acetic anhydride was added. The reaction mixtures were stirred for 16 h. The solvent was removed and the crude reaction product chromatographed to give the pure acetylated products.

4.4.5.1. (1*S*)-(−)-1-(4-Chlorophenyl)-2-phenylethyl acetate 9. White solid; mp 34–35°C; $[\alpha]_D^{25}=+5.3$ ($CHCl_3$, $c=3.2$ mg/ml):, 99% ee, IR $\nu_{max.}$ (film): 3063, 3029, 2925, 1739, 1235 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.01 (3H, s, CH_3CO-), 3.00 (1H, dd, $J=$ 13.7, 6.5 Hz, H-2a), 3.17 (1H, dd, $J=13.7$, 7.5 Hz, H-2b), 5.9 (1H, dd, $J=7.0$, 7.1 Hz, H-1), 7.06 (2H, d, $J=8.0$ Hz, H-2″, 6″), 7.17 (2H, d, $J=8.6$ Hz, H-2′, 6′), 7.20–7.26 (3H, m, H-3″, 5″, 4″), 7.26 (2H, d, $J=$ 8.4 Hz, H-3′, 5′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.12 (c, $\underline{C}H_3CO$), 42.80 (t, C-2), 75.96 (d, C-1), 126.69 (d, C-4″), 128.02 (d, C-2″, 6″), 128.30 (d, C-3″, 5″), 128.53 (d, C-2′, 6′), 129.49 (d, C-3′, 5′), 133.72 (s, C-4′), 136.51 (s, C-1′), 138.48 (s, C-1″), 170.01 (s, $CH_3\underline{C}O$); EIMS m/z: 216 (28), 214 (75), 179 (85) 178 (100); 141 (28), 125 (38), 91 (75). The enantiomeric excess was measured by means of chiral HPLC with *n*-hexane/*i*-PrOH 99.9:0.1 as eluent, flow rate 0.8 mL/min, t_R/min = 27.0 ((*S*)-**9**), 33.7 ((*R*)-**9**).

Acknowledgements

This work was supported by grants from DGICYT AGL2000-0635-C02-01 and AGL2002-04388-C02-01.

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