Chemoenzymatic Synthesis and Biological Activity of Both Enantiomeric Forms of Tetraconazole, a New Antifungal Triazole

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Both enantiomers of (*R,S*)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether (tetraconazole, **1**), a new, broad spectrum, triazole fungicide, were prepared by stereoselective lipase-catalyzed hydrolysis of the racemic precursor (*R,S*)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-prop-1-yl acetate (**4**) or by lipase-catalyzed transesterification of the prochiral precursor 2-(2,4-dichlorophenyl)-1,3-propanediol (**5**). The biological activity of both enantiomers has been investigated in vitro against a number of fungi and in vivo against *Sphaerotheca fuliginea* on cucumber and *Erysiphe graminis* on wheat. The *R*-(+) form has been found to be the more fungitoxic.

INTRODUCTION

(*R,S*)-2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether (tetraconazole, **1**) (Figure 1) is a new, broad spectrum, triazole fungicide, highly active against a number of *Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes*. It has recently been proposed as a new agent for crop protection (Garavaglia et al., 1988; Gozzo et al., 1989) and is being developed as the racemate.

The study of bioactive molecules has shown that biological activity and selectivity may often vary dramatically, both in degree and in kind, within a set of stereoisomers (Koller, 1987). In fact, a pair of enantiomers can generally be regarded as two different molecules from the biological point of view. It is therefore not surprising that also among the triazole fungicides, which inhibit ergosterol biosynthesis, stereoisomerism plays a very important role. It may determine fungitoxicity, effects on plant physiology, such as plant growth regulating (PGR) activity, metabolic fate in fungi and, possibly, mode of action and behavior inside the plants (Fuchs, 1988). Consequently, we had an interest in preparing both enantiomers of tetraconazole to compare their biological activities.

At the present time no simple chemical methods, however, exist for the synthesis of optically active isomers. The available chemical processes, in fact, are very complicated and require expensive chiral organic reagents. In recent years enzymatic catalysis has been used with success for the optical resolution of several classes of compounds (Chen and Sih, 1989; Francalanci et al., 1987; Jones, 1986; Whitesides and Wong, 1985). The simplicity of the operations, as well as the high yields and excellent stereoselectivities that can be achieved, makes this approach extremely attractive.

In this paper we report two strategies for the preparation of optically active **1** through the lipase-catalyzed, stereoselective hydrolysis of its racemic precursor (*R,S*)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-prop-1-yl acetate (**4**) (Figure 2) and through the asymmetric synthesis of optically active 2-(2,4-dichlorophenyl)-3-acetoxypropan-1-ol (**6**) by lipase-catalyzed transesterification of the corresponding diol **5** (Figure 3). Furthermore, the absolute configuration of (+) and (−) **1** were also assigned by chemical correlation of (*R*)-(−)-**6** with (*S*)-(−)-tropic acid.

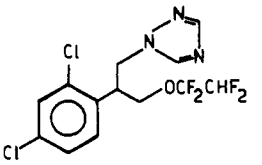

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Figure 1. Tetraconazole.

We also report the results of tests performed to investigate the in vitro fungitoxicity of the two enantiomers (Table III) and the in vivo activity against *Sphaerotheca fuliginea* on cucumber and *Erysiphe graminis* on wheat (Table IV). The in vivo tests provided on evaluation of the 1-day curative activity and the residual-protective translaminar activity (by treating 1 and 7 days before inoculations) on cucumber and of the 1-day curative and protective activity on wheat.

EXPERIMENTAL PROCEDURES

General Procedures. The optical rotation was measured with a Perkin-Elmer 241 polarimeter. ^{1}H NMR spectra were recorded in $CDCl_3$ or CD_3OD solution [$(CH_3)_4Si$ as internal standard] on a Bruker AM 300 instrument.

The optical purity of compound **6** was determined by ^{1}H NMR of the corresponding MTPA (Mosher acid) ester (Dale et al., 1969).

GLC analyses were carried out on a Carlo Erba HRGC 5300 with a 2 m × 4 mm SP 2100 3% column at 100–280 °C and with a flame ionization detector.

The optical purity of compound **3** was determined, on the corresponding MTPA ester, by using a 25 m × 0.32 mm OV 1 capillary column at 150–300 °C. Silica gel column chromatography was conducted on silica gel 60, 230–400 mesh ATSM (Merck).

Chemicals. Lipase Amano P from *Pseudomonas fluorescens* (30 units/mg) was purchased from Amano Chemical Co. Porcine pancreatic lipase (13 units/mg) and lipase from *Candida cylindracea* (500 units/mg) were purchased from Sigma Chemical Co. Pancreatine (57 units/mg) was purchased from Unibios.

All the organic chemicals used were purchased from Fluka Chemie.

(*R,S*)-2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-prop-1-yl Acetate. 2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-1-ol (8 g, 29.3 mmol) was added to acetic anhydride (100 mL) and refluxed for 10 h. The anhydride was removed in vacuo, and the residue was dissolved in ethyl acetate, washed with water, dried (Na_2SO_4), and evaporated to dryness. Chromatography on silica gel using ethyl acetate/hexane (7:3) as eluent afforded 7.9 g of (*R,S*)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-

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1-yl)prop-1-yl acetate: ^{1}H NMR ($CDCl_3$) δ 7.90 (1 H, s), 7.75 (1 H, s), 7.40–6.95 (3 H, m), 4.60–4.20 (4 H, m), 4.15–4.00 (1 H, m), 2.03 (3 H, s). Anal. Calcd for $C_{13}H_{13}Cl_2N_3O_2$: C, 49.70; H, 4.17; N, 13.37. Found: C, 49.25; H, 4.02; N, 13.40.

Enzymatic Hydrolysis of (*R,S*)-2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)prop-1-yl Acetate. To a magnetically stirred mixture of (*R,S*)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)prop-1-yl acetate (8 g, 25.4 mmol), dioxane (10 mL), and 0.05 M phosphate buffer (pH 7) (200 mL) at 35 °C was added porcine pancreatic lipase (2 g), and the pH was kept at 7.0 with 1 M aqueous sodium hydroxide by using a pH stat.

The hydrolysis was stopped at 50% conversion (24 h). The reaction mixture was extracted with ethyl acetate, and the organic layer was dried (Na_2SO_4) and evaporated to dryness. Chromatography on silica gel with ethyl acetate/hexane (7:3) as eluent afforded 3.5 g of (*S*)-(−)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)prop-1-yl acetate, $[\alpha]^{25}_D$ −12.9° (*c* 1, $CHCl_3$), and 2.8 g of (*R*)-(+)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-1-ol, ee ≥95%, $[\alpha]^{25}_D$ +45.0° (*c* 1, MeOH): ^{1}H NMR ($CDCl_3$) δ 7.91 (1 H, s), 7.88 (1 H, s), 7.42–7.20 (3 H, m), 4.62–4.43 (2 H, m), 3.95–3.70 (2 H, m), 2.98–3.10 (1 H, m), 1.78 (1 H, s). Anal. Calcd for $C_{11}H_{11}Cl_2N_3O$: C, 48.55; H, 4.07; N, 15.44. Found: C, 47.98; H, 3.95; N, 15.48.

Alkaline Hydrolysis of (*S*)-(−)-2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)prop-1-yl Acetate. (*S*)-(−)-2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)prop-1-yl acetate, $[\alpha]^{25}_D$ −12.9° (*c* 1, $CHCl_3$) (2.2 g, 7.0 mmol), was dissolved in a 1 M solution of sodium hydroxide in absolute ethanol (30 mL).

The solution was stirred for 2 h at 25 °C and then evaporated under vacuum. The residue was extracted with dichloromethane, and the organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness. Chromatography on silica gel with ethyl acetate/hexane (7:3) as eluent afforded 1.3 g of (*S*)-(−)2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-1-ol, ee ≥95%, $[\alpha]^{25}_D$ −45.1° (*c* 1, MeOH).

(*S*)-(−)-2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-propyl 1,1,2,2-Tetrafluoroethyl Ether. (*S*)-(−)-2-(2,4-Dichlorophenyl-3-(1*H*-1,2,4-triazol-1-yl)propan-1-ol (1.6 g, 5.8 mmol) was dissolved in a mixture of toluene (20 mL) and DMSO (3 mL). The solution was cooled to −5 °C, and finely ground potassium hydroxide (188 mg, 3.3 mmol) was added.

The air in the reaction flask was replaced with tetrafluoroethylene, and the solution was stirred for 2 h at −5 °C. The mixture was washed with ice–water, dried (Na_2SO_4), and evaporated to dryness.

Chromatography on silica gel with hexane/ethyl acetate (8:2) as eluent afforded 1.8 g of (*S*)-(−)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether, $[\alpha]^{25}_D$ −23.4° (*c* 1, MeOH): ^{1}H NMR ($CDCl_3$) δ 7.91 (1 H, s), 7.82 (1 H, s), 7.52–6.98 (3 H, m), 6.71 (1 H, tt), 4.68–4.40 (2 H, m), 4.33–4.05 (3 H, m). Anal. Calcd for $C_{13}H_{11}Cl_2F_4N_3O$: C, 41.96; H, 2.97; N, 11.29. Found: C, 42.21; H, 3.05; N, 11.96.

(*R*)-(+)-2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether, $[\alpha]^{25}_D$ +23.4° (*c* 1, MeOH) was synthesized according to the above described procedure, but starting from (*R*)-(+)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-1-ol.

Fractional Precipitation of Porcine Pancreatic Lipase. A centrifuged solution of crude porcine pancreatic lipase (10 g) in 0.02 M phosphate buffer (pH 8) (100 mL) was cooled to 5 °C, Celite 577 (25 g) was added, and acetone (100 mL) was dropped in 20 min at 5 °C.

The mixture was stirred at 5 °C for a further 30 min, and then the Celite with the adsorbed enzyme was filtered off and dried under vacuum until its water content was less than 1%.

Enzymatic Transesterification of 2-(2,4-Dichlorophenyl)-1,3-propanediol. To a solution of 2-(2,4-dichlorophenyl)-1,3-propanediol (7 g, 31.5 mmol) in anhydrous ethyl acetate (560 mL) was added porcine pancreatic lipase adsorbed on Celite as described above (24 g), and the suspension was shaken for 16 h at 25 °C. The enzyme was recovered by filtration, the solution was evaporated to dryness, and the residue was purified by chromatography on silica gel with hexane/ethyl acetate (7:3) as eluent, to afford 6.8 g of (*R*)-(+)-2-(2,4-dichlorophenyl)-3-acetoxypropan-1-ol, ee ≥95%, $[\alpha]^{25}_D$ +13.4° (*c* 1, $CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 7.40–7.18 (3 H, m), 4.50–4.25 (2 H, m), 3.83 (2 H, d), 3.73–3.60 (1 H, m), 2.07 (3 H, s). Anal. Calcd for $C_{11}H_{12}Cl_2O_3$: C, 50.21; H, 4.60. Found: C, 51.40; H, 4.55.

(*S*)-(+)-(2,4-Dichlorophenyl)-3-acetoxypropyl Toluene-4-sulfonate. To a solution of (*R*)-(+)-2-(2,4-dichlorophenyl)-3-acetoxypropan-1-ol (3.1 g, 11.7 mmol) in pyridine (10 mL) was added toluene-4-sulfonyl chloride (2.7 g, 14.2 mmol) in small portions at 5 °C.

The mixture was stirred for 6 h at room temperature and then diluted with water, acidified with 1 M hydrochloric acid at 5 °C, and extracted with ethyl acetate. The organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was crystallized from hexane/diethyl ether (1:1), affording 3.6 g of (*S*)-(+)-2-(2,4-dichlorophenyl)-3-acetoxypropyl toluene-4-sulfonate with $[\alpha]^{25}_D$ +5.2° (*c* 1, $CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 7.74–7.00 (7 H, m), 4.40–4.15 (4 H, m), 3.87–3.70 (1 H, m), 2.45 (3 H, s), 1.98 (3 H, s). Anal. Calcd for $C_{18}H_{18}Cl_2SO_5$: C, 51.81; H, 4.35. Found: C, 52.20; H, 4.11.

(*R*)-(+)-2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-prop-1-yl Acetate. The sodium salt of 1,2,4-triazole (840 mg, 9.2 mmol) was added to a solution of (*S*)-(+)-2-(2,4-dichlorophenyl)-3-acetoxypropyl toluene-4-sulfonate (3.1 g, 7.5 mmol) in DMF (30 mL), and the mixture was stirred for 3 h at 60 °C.

The solvent was evaporated under vacuum, and the residue was dissolved in ethyl acetate, filtered, and evaporated to dryness.

Chromatography on silica gel with ethyl acetate as eluent afforded 1.8 g of (*R*)-(+)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-prop-1-yl acetate, ee ≥95%, $[\alpha]^{25}_D$ +12.4° (*c* 1, $CHCl_3$).

(*R*)-(+)-2-Phenyl-3-acetoxypropan-1-ol. Pd on charcoal (1.6 g) was added to a solution of (*R*)-(+)-2-(2,4-dichlorophenyl)-3-acetoxypropan-1-ol (4 g, 15.1 mmol) and *N,N*-diisopropylethylamine (5.6 mL, 32.1 mmol) in anhydrous tetrahydrofuran (200 mL). The mixture was shaken in H_2 atmosphere for 48 h at room temperature, diluted with ethyl acetate, and filtered on Celite.

The solvent was evaporated, and the residue was purified by chromatography on silica gel with ethyl acetate/hexane (1:1) as eluent affording 2.4 g of (*R*)-(+)-2-phenyl-3-acetoxypropan-1-ol with $[\alpha]^{25}_D$ +15.6° (*c* 1, $CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 7.40–7.18 (5 H, m), 4.40 (2 H, d), 3.84 (2 H, d), 3.22–3.06 (1 H, m), 2.05 (3 H, s), 1.81 (1 H, s). Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.26. Found: C, 69.00; H, 7.81.

(*S*)-(−)-2-Phenyl-3-acetoxypropionic Acid. A solution of chromic anhydride (4.5 g, 45 mmol) in water (12 mL) was carefully mixed with 96% sulfuric acid (4.5 mL) at 5 °C, and the resulting mixture was added dropwise to a solution of (*R*)-(+)-2-phenyl-3-acetoxypropan-1-ol (3 g, 15 mmol) in acetone (400 mL) at 0 °C. After stirring for 1 h at 0 °C, the salts were filtered off and the solvent was evaporated to give a residue, which was dissolved in dichloromethane, washed with water, dried (Na_2SO_4), and evaporated to dryness. Chromatography on silica gel with hexane/ethyl acetate (8:2) as eluent afforded 1 g of (*S*)-(−)-2-phenyl-3-acetoxypropionic acid with $[\alpha]^{25}_D$ −52.3° (*c* 1, $CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 11.58 (1 H, s), 7.47 (5 H, m), 4.85–3.88 (3 H, m), 2.01 (3 H, s). Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 64.02; H, 5.60.

(*S*)-(−)-Tropic Acid. (*S*)-(−)-2-Phenyl-3-acetoxypropionic acid (1 g, 4.8 mmol) was dissolved in 1 M potassium hydroxide in methanol (10 mL). The solution was stirred for 4 h, the solvent was evaporated, and the residue was dissolved in water and washed with diethyl ether.

The aqueous phase was acidified to pH 5 with 1 M hydrochloric acid and extracted with dichloromethane. The organic phase was dried (Na_2SO_4) and evaporated, and the residue was crystallized from benzene to afford 300 mg of (*S*)-(−)-tropic acid with $[\alpha]^{25}_D$ −71.8° (*c* 1, water) [lit. (Fodor and Csepreghy, 1961) $[\alpha]^{25}_D$ −72.0° (*c* 1, water)]: ^{1}H NMR (CD_3OD) δ 7.40–7.16 (5 H, m), 4.08 (1 H, t), 3.80–3.64 (2 H, m). Anal. Calcd for $C_9H_{10}O_3$: C, 65.05; H, 6.06. Found: C, 65.91; H, 5.99.

Bioassay. *In Vitro Activity.* Fungitoxicity tests were carried out in Petri dishes, with potato dextrose agar, by assessing the inhibition of mycelial radial growth of treated colonies with respect to untreated ones.

Activity on Plants. Activity against *S. fuliginea* was evaluated on artificially infected, potted, cucumber plants (cv. Marketer). Infections were carried out by spraying the leaves with a spore suspension *S. fuliginea* in water (200.000 conidia/mL). Curative

Table I. Enzymatic Hydrolysis of (*R,S*)-4[a]

enzyme	time, h	conv, %	alcohol 3 $[\alpha]^{25}$[b]	alcohol 3 conf[c]	alcohol 3 ee,[d] %	ester 4 $[\alpha]^{25}$[e]	ester 4 conf	ester 4 ee,[f] %
PPL	24	50	+45.0	*R*	≥95	−12.9	*S*	≥95
pancreatine	10	50	+39.5	*R*	86	−9.4	*S*	70
lipase P	18	75	−2.8	*S*	6	+3.0	*R*	22
CCY	30	30	−8.7	*S*	19	+1.2	*R*	9

[a] All the reactions were performed in phosphate buffer (pH 7), 0.05 M (200 mL), and dioxane (6 mL) at 35 °C; substrate, 25 mmol; lipase, 2 g. [b] *c* 1, MeOH. [c] Determined on the basis of the specific rotation of *R*-(+)-3 obtained from *R*-(+)-6. [d] The enantiomeric excess (ee) was determined by GLC analysis of the corresponding α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) ester (Dale et al., 1969). [e] *c* 1, $CHCl_3$. [f] The enantiomeric excess (ee) was determined on the basis of the optical purity of the alcohol obtained from the ester by alkaline hydrolysis.

Table II. Enzymatic Transesterification of 5[a]

enzyme	amount of enzyme, mg/mL	time, h	conv, %	$[\alpha]^{25}$[b]	ester 6 conf[c]	ee,[d] %
lipase P	4	15	80	+10.9	*R*	81
CCY	75	70	80	+3.2	*R*	24
PPL	35	15	70	+7.2	*R*	53
PPL/Celite[e]	40	8	95	+12.8	*R*	95
PPL/Celite[f]	40	16	95	+13.4	*R*	99

[a] All the reactions were performed in ethyl acetate (10 mL) at 25 °C; substrate, 2.3 mmol. [b] *c* 1, $CHCl_3$. [c] Determined by correlation with (*S*)-tropic acid. [d] The enantiomeric excess (ee) was determined by ^1H NMR of the corresponding MTPA ester (Dale et al., 1969). [e] 10 g of crude enzyme precipitated on 25 g of Celite 577 with 60% acetone in phosphate buffer (pH 8). [f] Same procedure as in (*e*) but using 50% acetone.

treatments were carried out 1 day after artificial infection. Preventive treatments were carried out 1 or 7 days before infection; translaminar activity was evaluated by treating the lower leaf surface and then inoculating the upper one.

Cucumber plants were treated by sprinkling the leaves with the compounds dissolved in a water/acetone solution containing 20% acetone (v/v) and 0.03% Tween 20. Inoculated plants were kept in a conditioned room with a daylight period of 16 h and relative humidity 70% for 12 days at 20 °C to incubate *S. fuliginea*.

At the end of the incubation period, fungicidal activity was assessed as percent disease control with respect to infected but untreated plants.

RESULTS AND DISCUSSION

Racemic ester 4 was prepared in high yields according to the method of Colle et al. (1987). Methyl 2-(2,4-dichlorophenyl)-3-hydroxypropionate (2), obtained by conventional methods, was efficiently transformed into the corresponding triazole derivative via its mesylate. Subsequent reduction with $LiAlH_4$ afforded alcohol 3, which was treated with acetic anhydride, and the resulting acetate 4 was used in the enzymatic hydrolysis.

Several commercially available hydrolytic enzymes were tested, but only two lipase preparations, porcine pancreatic lipase and pancreatin, gave satisfactory results.

Enzymatic hydrolyses were carried out at pH 7 and 35 °C. The pH was kept constant by addition of 0.1 M aqueous NaOH. The reactions were stopped at different degrees of conversion, and the products were recovered as described under Experimental Procedures.

The optical purity of 3 was determined by GLC of the MTPA (Mosher acid) esters.

As shown in Table I, in the hydrolytic reaction the two mammalian lipases PPL and pancreatin were more stereoselective than those from microorganisms. The alcohol (*R*)-(+)-3 and the ester (*S*)-(−)-4, products of the enzymatic hydrolysis, were separated by chromatography on silica gel and transformed in (*R*)-(+)-1 and (*S*)-(−)-1 as shown in Figure 2.

Table III. EC_{50} Values (Milligrams per Liter) of Tetraconazole Racemate and Its Enantiomers in Fungicidal in Vitro Tests

pathogen	racemate	*R*-(+)	*S*-(−)	enantiomeric activity ratio (*R*/*S*)
Botrytis cinerea	2.1	1.2	23.5	19.6
Cercospora beticola	0.45	0.24	2.3	9.6
Cladosporium cucumerinum	0.17	0.11	0.85	7.7
Guignardia bidwellii	0.035	0.02	0.12	6.0
Helminthosporium gramineum	1.09	0.54	1.16	2.1
Helminthosporium maydis	0.13	0.1	0.29	2.9
Helminthosporium oryzae	5.5	3.5	6.8	1.9
Helminthosporium sativum	1.7	0.8	2.4	3.0
Helminthosporium teres	0.3	0.18	0.64	3.5
Pyricularia oryzae	2.1	1.5	2.8	1.9
Sclerotinia minor	2.1	1.4	9.0	6.4
Sclerotium cepivorum	0.3	0.15	1.9	12.7

In the second approach, the possibility of preparing optically pure 1 by enantioselective enzymatic transformation of a prochiral precursor was investigated. 2-(2,4-Dichlorophenyl)-1,3-diacetoxypropane, prepared by conventional methods, was submitted to lipase-catalyzed stereoselective hydrolysis to produce the optically active monoester 6. However, further extensive enzymatic hydrolysis occurred to give the diol 5 (approximately 70%), a side reaction that could not be suppressed by any change in the experimental conditions.

Lipase-catalyzed transesterification reaction of diol 5 in organic solvent, instead, afforded the monoester 6 in high yields and purity. The transesterification reactions were carried out in ethyl acetate, which acted as both acylating agent and reaction medium. The enzymes were used either in powdered form or supported on Celite. Periodically, 1-μL aliquots were drawn to be analyzed by gas chromatography. The reaction mixtures were worked up as described under Experimental Procedures. The optical purity of 6 was determined by ^1H NMR of the corresponding MTPA (Mosher acid) ester.

Several commercially available hydrolytic enzymes were tested, but only one of animal origin (porcine pancreatic lipase) and one of microbial origin (lipase Amano P from *Pseudomonas fluorescens*) proved to be effective catalysts (Table II). Moreover, the optical purity of the recovered products was not satisfactory for our purposes.

Since the commercially available PPL preparations are a mixture of enzymatic and water-soluble nonenzymatic materials, it was decided to purify this crude preparation. The catalyst for transesterification was prepared according to the method of Tombo et al. (1986) by fractional precipitation of a PPL solution in phosphate buffer (pH 8) with acetone and adsorbing the precipitated proteins on Celite. The catalytic activity of this new enzymatic preparation was as good as that of crude PPL, but the stereoselectivity was much higher. As shown in Table II, the enantiomeric excess changed from 53% to 95%. Since the presence of water in the reaction mixture significantly inhibited the enzymatic activity, anhydrous organic solvents were used and the amount of water associated with the immobilized enzyme preparation was kept below 1%. Enzymatically prepared, optically active monoacetate 6 was successively transformed in (*R*)-(+)-1 by the sequence of reactions shown in Figure 3.

Absolute configurations of (+)- and (−)-1 were determined by chemical correlation with (*R*)-(+)-2-(2,4-dichlorophenyl)-3-acetoxypropan-1-ol (6), whose configuration

Table IV. EC_{90} Values (Milligrams per Liter) of Tetraconazole Racemate and Its Enantiomers in Fungicidal in Vivo Tests

pathogen/plant	treatment	racemate	R-(+)	S-(-)	enantiomeric activity ratio (R/S)
S. fuliginea/cucumber	cur.[a] 1 day	1.8	0.45	7.5	16.7
S. fuliginea/cucumber	prev[b] 1 day	7.5	1.8	30	16.7
S. fuliginea/cucumber	prev[b] 7 days	85	30	160	5.3
E. graminis/wheat[c]	cur.[a] 1 day	0.059	0.027	3.6	133
E. graminis/wheat[c]	prev[d] 1 day	0.40	0.20	80	400

[a] Curative application. [b] Translaminar protective application. [c] Data reprinted from Gozzo et al. (1989). [d] Protective application.

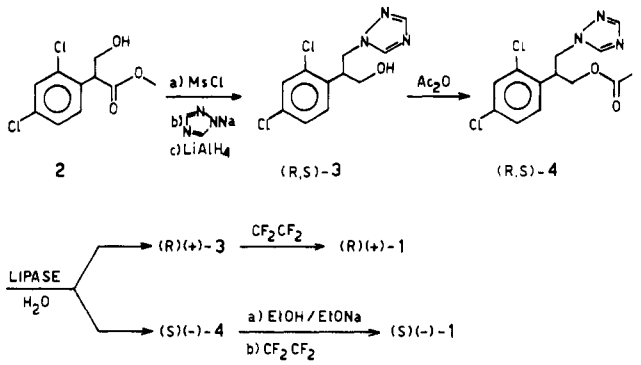

Figure 2. Synthetic route to both (*R*)- and (*S*)-tetraconazole by enzymatic resolution of (*R*,*S*)-4.

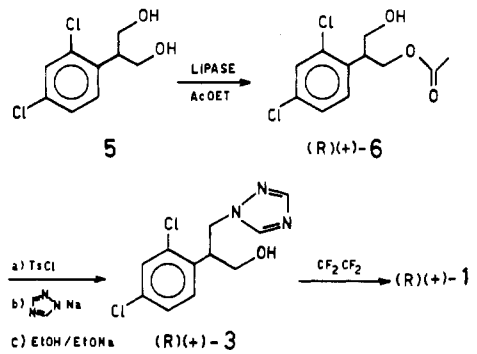

Figure 3. Synthetic route to (*R*)-tetraconazole by enzymatic synthesis of (*R*)-(+)-6.

was in its turn related to that of (*S*)-(−)-tropic acid (see Experimental Procedures).

The fungicidal activity of the racemate and of the two enantiomers has been assessed both in vitro, against a number of fungi (Table III), and on cucumber plants, against *S. fuliginea* (Table IV).

The in vitro tests show that susceptibility may differ by at least 2 orders of magnitude among the various fungi and that the (*R*)-(+) isomer is always the more active. There is no relationship between susceptibility to the more active isomer and the activity ratio of the enantiomers. The ED_{50} values of the racemate show that the *S*-(−) form does not significantly contribute to the fungicidal activity in all but a few cases, when a modest, additive effect is noticed.

The tests on plants confirm the greater activity of the *R*-(+) form. The results obtained in the control of *S. fuliginea* on cucumber plants reflect the usually higher rates needed in the protective treatments with respect to the curative ones. This is a general rule for the systemic EBIs. However, the data on *S. fuliginea* differ from these that we have already reported (Gozzo et al., 1989) on the control of another powdery mildew, wheat *E. graminis*, by the same compounds and that are shown in Table IV for an easier comparison.

The curative activity ratios of the two enantiomers are found to be high in the case of *E. graminis* and low in the case of *S. fuliginea*. The activity ratios of the racemate and of the *R*-(+) enantiomer equal 2 for *E. graminis* and 4 for *S. fuliginea*.

On cucumber, this latter, anomalously high ratio would apparently indicate a measure of antagonism between the two enantiomers, but may also suggest interference by the plant.

In comparison with the tests on wheat described previously (Gozzo et al., 1989), where the isomers were applied as preventive treatment on the whole leaf surface and might thus interfere with the pathogen directly, in the present work the enantiomers, once applied on cucumber leaves, must penetrate, move across and persist in the foliar tissues before they can reach the pathogen.

The results of the translaminar application tests on cucumber indicate tht the systemic properties of the two enantiomers do not differ appreciably. However, the 7-day preventive activity tests, where the activity ratio is modified in favor of the *S*-(−) isomer, show that the plant may exert some selective effect on the behavior of the enantiomers.

Differential metabolism and/or compartmentalization may affect the intrinsic activities of the enantiomers and explain the behavior observed. It would be interesting to find out if this strictly depends on the type of application or on the host/pathogen relationship.

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