Synthesis, Fungicidal Activity, and QSAR of a Series of 2-Dichlorophenyl-3-triazolylpropyl Ethers

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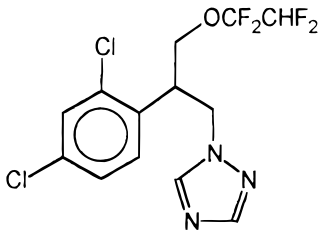
A series of new alkyl and arylalkyl ethers of 2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propanol, related to the fungicide tetraconazole, were synthesized and tested in vitro or in vivo against seven common pathogens in comparison with tetraconazole. In vitro, most of them exhibited a broad spectrum of activity and an efficacy of the same order of magnitude of the standard, but the activity was influenced by the nature of the substituents. A QSAR study showed that lipophilicity is a major positive parameter in affecting the activity; the second relevant parameter is μ, whereas geometrical descriptors indicate that linear and narrow substituents are more suitable than wide ones. In in vivo assays some compounds had good activity on bean rust, either protective or curative. Sterol analysis showed that the mechanism of action is due to inhibition of 14α-demethylase.

Keywords: *Fungicides; triazoles; sterol biosynthesis inhibitors; QSAR*

INTRODUCTION

Azole fungicides have been used since the early 1970s for the protection of various crops. The introduction of these sterol biosynthesis inhibitors represented significant progress in the chemical control of fungal diseases. They include several excellent systemic fungicides with long protective and curative activity against a broad spectrum of foliar, root, and seedling diseases caused by many ascomycetes, basidiomycetes, and imperfect fungi (Berg, 1986).

Among these, tetraconazole [M-14360, (±)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-propyl 1,1,2,2-tetrafluoroethyl ether, **1**] is characterized by a higher



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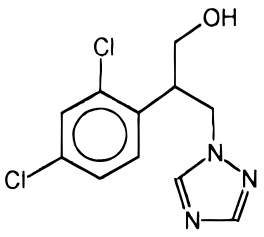
efficacy against biotrophes than most commercial analogues, with respect to which, however, it is generally less active when assayed in vitro on the inhibition of mycelial growth of many necrotrophes (Gozzo et al., 1990; Carzaniga et al., 1991a). Structurally, tetraconazole differs from many other azole fungicides (Zirngibl, 1998) for the type of bridge between the aromatic and the triazole rings, that is, a two-carbon chain with a primary alkoxy group.

Although the mechanism of action of tetraconazole has been studied in detail (Carzaniga et al., 1991a; Carelli et al., 1992; Gozzo et al., 1995 and preceding papers quoted therein), very little is known about the structure−activity relationship in tetraconazole analogues. The present paper reports the synthesis, the in vitro and in vivo fungicidal activities, and a quantitative structure−activity study of a new series of alkyl and arylalkyl ethers related to tetraconazole.

2-(2,4-Dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propanol, **2**, was considered to be a suitable key intermedi-



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ate for the synthesis of such compounds, the structures of which are presented in Figure 1. The ethers **3** contain a benzyl group bearing various substituents: the length of these substituents was systematically increased to explore the size of the receptor site. Ether **3n** contains a thienyl group, which is considered to be isosteric with the phenyl group.

The ethers **4** contain alkyl chains with a double bond (**4a**−**d**), a triple bond (**4e**), a phenyl group (**4f,g**), or a polar group (**4h,i**).

As all compounds are chiral, because they contain a stereogenic carbon atom, one derivative of class **3** and one of class **4** were selected to prepare single enantiomers.

Alkene **5** was detected sometimes as a reaction byproduct especially during attempts to convert alcohol **2** into the sulfonate or halide, due to an easy elimination

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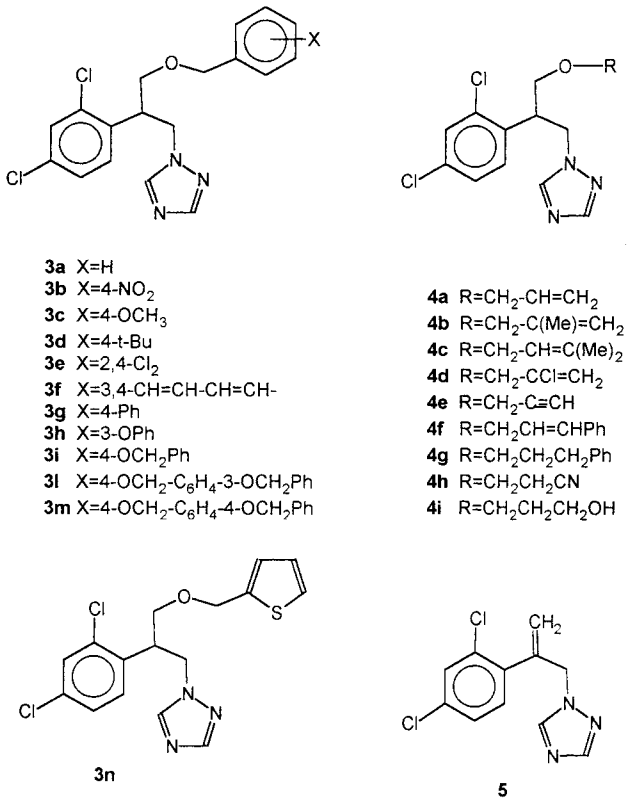


Figure 1. Structures of the compounds that were synthesized and tested.

of these good leaving groups. This behavior raised the suspicion that ethers **3** and **4** could undergo an in vivo elimination reaction, thus all producing **5** as the biologically active compound. Therefore, this compound was prepared to assess its fungicidal activity.

MATERIALS AND METHODS

Chemistry. Melting points are uncorrected. Dry solvents were prepared according to usual methods. Column chromatographies were performed on silica gel 60, 230−400 mesh ATSM (Merck). Optical rotations were measured with a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on a Bruker WP80 at 80 MHz or on a Varian XL300 instrument at 300 MHz, using tetramethylsilane as internal standard. Mass spectra were recorded on a Finnigan-MAT TSQ70 spectrometer. Infrared spectra were recorded with a Perkin-Elmer 1310 spectrophotometer. All of the compounds synthesized had satisfactory analytical data.

Porcine pancreatic lipase (PPL; 2.4 units/mg) was purchased from Fluka. Alcohol **2** was kindly provided by Isagro (Milano, Italy).

Alcohols *R*-(+)-**2** ($[\alpha]_D = +46$ (*c* 1 MeOH); lit. $[\alpha]_D = +45$) and *S*-(−)-**2** ($[\alpha]_D = -44$ (*c* 1 MeOH); lit. $[\alpha]_D = -45.1$) were prepared by enantioselective hydrolysis of the racemic acetate by PPL according to a procedure of Bianchi et al. (1991).

Procedure A. General Methodology for the Synthesis of Compounds 3 and 4. The alcohol **2** (2 mmol), the appropriate alkyl bromide or chloride (4 mmol), and tetrabutylammonium bromide (0.2 mmol) were dissolved in dichloromethane (8 mL). An aqueous solution of NaOH (3 mmol, 8 mL of water) was added, and the mixture was stirred under reflux; ether formation was monitored by TLC. After separation of the two phases and extraction of the aqueous one with dichloromethane (2 × 5 mL), the combined organic extracts were dried and concentrated. The desired compounds were purified by flash column chromatography on silica gel using hexane/ethyl acetate as eluent. Yields and melting points are collected in Table 1. Examples of the spectroscopic characteristics of the compounds: **3a**, NMR ($CDCl_3$) δ 3.6 (2 H, m, CH_2-Triaz), 4.06 (1 H, m, CH), 4.50 (2 H, s, $PhCH_2O$), 4.58 (2 H, m, CH_2O), 7.1−7.5 (8 H, m, arom), 7.78 and 7.90 (2 H, 2 s, triazole); MS, *m*/*z* (%) 361 (M^+, 4), 326 (23), 270 (26), 255 (31), 188 (41), 186 (64), 172 (100), 159 (25). **4a** NMR ($CDCl_3$) δ 3.6 (2 H, m, CH_2-Triaz), 4.0 (3 H, CH and CH_2O), 4.55 (2 H, m, CH_2O), 5.0−5.4 (2 H, m, $=CH_2$), 5.6−6.1 (1 H, m, CH=), 7.1−7.4 (3 H, m, arom), 7.91 and 7.96 (2 H, 2 s, triazole); MS, *m*/*z* (%) 311 (M^+, 42), 276 (92), 270 (80), 254 (30), 204 (58), 175 (34), 172 (100), 159 (50), 137 (25).

Table 1. Synthesized Compounds and Their Chemical Characteristics

					mass spectrometry	
compd	method	yield	mp (°C)	formula	molecular peak (%)	base peak
3a	A	67	oil	$C_{18}H_{17}Cl_2N_3O$	361 (4)	172
3b	A	93	102	$C_{18}H_{16}Cl_2N_4O_3$	406 (5)	172
3c	A	10	oil	$C_{19}H_{19}Cl_2N_3O_2$	391 (4)	121
3d	A	30	oil	$C_{22}H_{25}Cl_2N_3O$	391 (7)	186
3e	A	64	56−58	$C_{18}H_{15}Cl_4N_3O$	431 (4)	159
3f	A	37	oil	$C_{22}H_{19}Cl_2N_3O$	411 (5)	70
3g	A	66	oil	$C_{24}H_{21}Cl_2N_3O$	437 (2)	70
3h	A	19	oil	$C_{24}H_{21}Cl_2N_3O_2$	453 (12)	70
3i	A	52	oil	$C_{25}H_{23}Cl_2N_3O_2$	467 (5)	91
3l	A	14	oil	$C_{32}H_{29}Cl_2N_3O_3$	573 (<1)	197
3m	A	12	oil	$C_{32}H_{29}Cl_2N_3O_3$	573 (9)	197
3n	A	13	oil	$C_{16}H_{15}Cl_2N_3OS$	367 (2)	111
4a	A	79	oil	$C_{14}H_{15}Cl_2N_3O$	311 (42)	172
4b	A	54	oil	$C_{15}H_{17}Cl_2N_3O$	325 (8)	172
4c	A	16	oil	$C_{16}H_{19}Cl_2N_3O$	339 (6)	69
4d	A	16	oil	$C_{14}H_{14}Cl_3N_3O$	345 (3)	172
4e	A	12	oil	$C_{14}H_{13}Cl_2N_3O$	309 (7)	274
4f	A	32	oil	$C_{20}H_{19}Cl_2N_3O$	387 (6)	70
4g	B	48	oil	$C_{20}H_{21}Cl_2N_3O$	389 (4)	118
4h	C	98	40	$C_{14}H_{14}Cl_2N_4O$	324 (5)	289
4i	D	30	86−87	$C_{14}H_{18}Cl_2N_3O_2$	331 (10)	172
5	E	20	oil	$C_{11}H_9Cl_2N_3$	253 (12)	218

Procedure B. Synthesis of Compound 4g. Compound **4f** (0.50 g, 1.2 mmol) in ethanol was treated with hydrogen in the presence of 10% Pd/C (0.12 g). After 6 h, the solution was filtered, evaporated, and chromatographed with hexane/ethyl acetate 9:1 to give the pure compound **4g** as an oil (0.24 g, 48% yield): NMR ($CDCl_3$) δ 1.98 (2 H, m, CH_2), 2.70 (2 H, m, CH_2Ar), 3.45 (2 H, t, $J = 7$ Hz, CH_2O), 3.58 (2 H, m, CH_2-Triaz), 4.05 (1 H, m, CH), 4.55 (2 H, s, $PhCH_2O$), 7.0−7.5 (8 H, m, arom), 7.93 and 7.98 (2 H, 2 s, triazole); MS, *m*/*z* (%) 389 ($M^{\bullet+}$, 4), 285 (10), 174 (24), 171 (41), 136 (11), 118 (100).

Procedure C. Synthesis of Compound 4h. A solution of alcohol **2** (3 g, 11 mmol), Triton-B (0.018 g, 1.1 mmol), and acrylonitrile (0.4 mL, 11 mmol) was stirred at room temperature for 15 min and then diluted with water (20 mL) and extracted with dichloromethane (3 × 30 mL). The organic layer was dried and evaporated to give 3.76 g (98% yield) of **4h** as an oil, which solidified slowly, mp 40 °C: IR, cm^{-1} 2310; NMR ($CDCl_3$) δ 2.64 (2 H, t, $J = 7$ Hz, CH_2CN), 3.5−3.9 (4 H), 4.08 (1 H, m, CH), 4.58 (2 H, AB of ABX, CH_2O), 7.1−7.6 (3 H, arom), 7.98 and 8.00 (2 H, 2 s, triazole); MS, *m*/*z* (%) 325 (M^+, 5), 291 (32), 290 (16), 289 (100), 172 (57), 159 (19).

Procedure D. Synthesis of Compound 4i. 9-Borabicyclononane [4 mmol, 8 mL of 0.5 M solution in tetrahydrofuran (THF)] was added to a solution of the alkene **4a** (0.45 g, 1.45 mmol) in anhydrous THF (14 mL) at −78 °C. The temperature was allowed to rise to 20 °C in 2 h, and then the mixture was stirred at the same temperature overnight. After cooling at 0 °C, the following reagents were added in sequence: ethanol (2.7 mL), NaOH (1.4 mL, 3 M), and 30% H_2O_2 (1.4 mL). The mixture was allowed to reach 20 °C, stirred for 15 min, and then poured into ethyl ether (200 mL). NaOH (50 mL, 1 M) and NH_4Cl (50 mL, saturated solution) were added. The organic phase was then washed with water, dried, and evaporated to give a yellowish oil, which was chromatographed with ethyl acetate as eluent to give compound **4i** as a colorless

solid (0.30 g, 30% yield): mp 86−87 °C; NMR ($CDCl_3$) δ 1.88 (2 H, m, CH_2), 3.5−3.8 (6 H), 3.95 (1 H, m, CH), 4.53 (2 H, m, CH_2O), 7.13 (1 H, d, J = 8 Hz, H-6′), 7.19 (1 H, dd, J = 8 and Hz, H-5′), 7.4 (1 H, d, J = 2 Hz, H-3′), 7.88 and 7.94 (2 H, 2 s, triazole); MS, *m*/*z* (%) 331 (M^+, 10), 329 (18), 294 (21), 174 (61), 172 (100), 137 (15).

Procedure E. Synthesis of Compound 5. A solution of methylmagnesium iodide obtained by treating Mg (0.7 g, 29 mmol) with methyl iodide (1.8 mL, 29 mmol) in anhydrous ethyl ether (30 mL) was added dropwise with 2,4-dichloroacetophenone (5 g, 26.4 mmol) in ethyl ether (20 mL); the temperature was maintained under 20 °C with a cool bath. The mixture was then refluxed for 7 h, cooled, and treated with NH_4Cl (3 g in 5 mL of water). After extraction with ethyl ether (2 × 30 mL), the organic phase was dried, evaporated, and purified by column chromatography (hexane/ethyl acetate 9:1) to give 2-(2,4-dichlorophenyl)propan-2-ol, **11**, as a colorless oil (3.5 g, 65% yield): NMR ($CDCl_3$) δ 1.73 (6 H, s, CH_3), 2.45 (OH), 7.20 (1 H, d, J = 2 Hz, H-3′), 7.38 (1 H, dd, J = 9 and 2 Hz, H-5′), 7.68 (1 H, d, J = 9 Hz, H-6′). This compound was heated at reflux for 5 h in acetic anhydride (10 mL). The mixture was poured into water (15 mL) and extracted with ethyl acetate. The organic phase was dried and evaporated, and the residue was purified by column chromatography to give 2-(2,4-dichlorophenyl)-2-acetoxypropane, **12** (2.38, 56% yield): NMR ($CDCl_3$) δ 1.83 (6 H, s, CH_3), 2.05 (3 H, s, $COCH_3$), 7.2−7.5 (3 H, arom). This compound (1.1 g, 4.5 mmol) was distilled bulb to bulb at 200 °C to give 2-(2,4-dichlorophenyl)-prop-1-ene, **13**, as a colorless oil (0.8 g, 95% yield): NMR ($CDCl_3$) δ 2.06 (3 H, m, CH_3), 4.98 (1 H, m, C=CH), 5.23 (1 H, m, C=CH), 7.0−7.4 (3 H, arom). This compound (0.8 g, 4.3 mmol) in 1,1,2,2,-tetrachloroethane (8 mL) was treated with *N*-bromosuccinimide (0.91 g, 5.1 mmol) and a catalytic amount of dibenzoylperoxide. The mixture was heated under reflux for 4 h and then filtered. The solvent was evaporated, and purification by column chromatography gave 0.81 g of a 57:43 mixture of 3-bromo-2-(2,4-dichlorophenyl)prop-1-ene, **15**, and 1-bromo-2-(2,4-dichlorophenyl)prop-1-ene, **14**. This mixture (0.50 g) was added with anhydrous triethylamine (0.2 mL, 2.82 mmol) and 1,2,4-triazole (0.25 g, 3.76 mmol) in ethyl acetate (15 mL). The mixture was heated at reflux for 40 h, then treated with HCl (1 M, 10 mL), and extracted with ethyl acetate (3 × 10 mL). The organic layer was washed with NaOH (1 M), dried and evaporated. Compound **5** was purified by column chromatography (220 mg, 47%); NMR ($CDCl_3$) δ 5.15 (2 H, s, CH_2-triaz), 5.31 (1 H, s, =CH), 5.45 (1 H, s, =CH), 7.00 (1 H, d, J = 8 Hz, H-6), 7.20 (1 H, dd, J = 8 Hz, H-6), 7.20 (1 H, dd, J = 8 and 2 Hz, H-5), 7.44 (1 H, J = 2 Hz, H-3), 7.95 and 8.00 (2 H, 2 s, triazole); MS, *m*/*z* (%) 253 (M^+, 12), 220 (29), 218 (100), 136 (11).

Synthesis of 4-[4-(Benzyloxy)benzyloxy]benzylbromide, 10a. 4-Benzyloxybenzyl chloride, **7a** (2.1 g, 9.02 mmol), methyl 4-hydroxybenzoate (1.37 g, 9.02 mmol), and anhydrous potassium carbonate (3.11 g, 22.5 mmol) were stirred overnight at room temperature in 2-butanone (90 mL) and then heated for 6 h. The solids were filtered, and the solvent was evaporated in part. The mixture was partitioned in 0.1 N HCl and ethyl acetate and then extracted with ethyl acetate. After drying, the solvent was evaporated to give an oil, which was purified by column chromatography giving 1.62 g (52% yield) of methyl ester **8a**: mp 137 °C; NMR ($CDCl_3$) δ 3.90 (3 H, s, OCH_3), 5.02 (2 H, s, OCH_2), 5.07 (2 H, s, OCH_2), 6.8−7.5 (11 H), 8.0 (2 H, m). This compound (1.62 g, 4.65 mmol) in THF (40 mL) was dropped in 25 min in a solution of $LiAlH_4$ (6.5 mL, 1 M solution in THF) in THF (38 mL). The mixture was heated at reflux for 4 h, ethyl acetate (1 mL) was added, and then 5% H_2SO_4 was added until pH 1 was obtained. The organic layer was separated, and the aqueous one was extracted with ethyl acetate. The combined extracts were dried and evaporated to give alcohol **9a** pure enough for the subsequent step (1.30 g, 88% yield, mp 124−126 °C): NMR ($CDCl_3$) δ 4.62 (2 H, s, CH_2OH), 4.98 (2 H, s, OCH_2), 5.07 (2 H, s, OCH_2), 6.8−7.5 (13 H). This compound (1.30 g, 4.06 mmol) was dissolved in dichloromethane (45 mL) and cooled at 0 °C, and then CBr_4 (1.99 g, 6.0 mmol) and triphenylphosphine (1.57 g, 6.0 mmol) were added in small portions in 30 min. The mixture was stirred at room temperature overnight and filtered. The solvent was evaporated, and the crude residue was purified by column chromatography. The title compound **10a** was obtained as a white solid: 0.86 g, 55%, mp 122−125 °C; NMR ($CDCl_3$) δ 4.50 (2 H, s, CH_2Br), 4.98 (2 H, s, OCH_2), 5.07 (2 H, s, OCH_2), 6.8−7.5 (13 H).

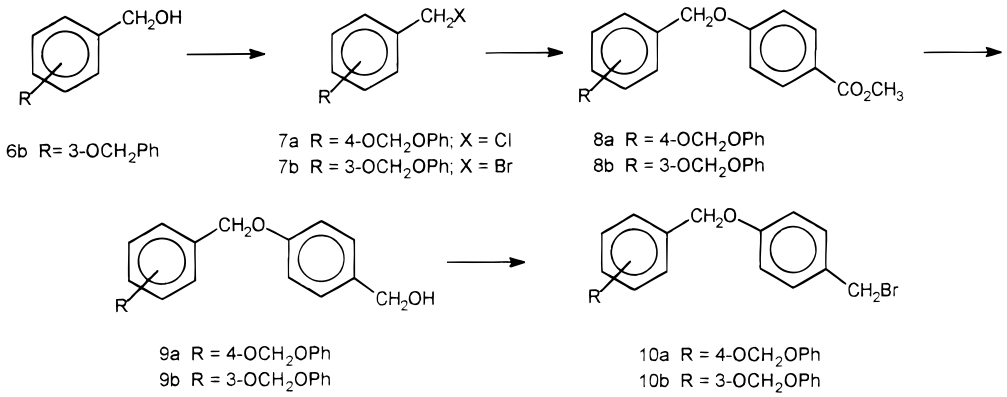
Synthesis of 4-[3-(Benzyloxy)benzyloxy]benzylbromide, 10b. Alcohol **6b** (1.34 g, 6.25 mmol) was dissolved in dry dichloromethane (45 mL) at 0 °C. Tetrabromomethane (3.11 g, 9.37 mmol) and triphenylphosphine (2.46 g, 9.37 mmol) were added, and the mixture was kept at room temperature for 22 h. The mixture was then filtered with suction, dried, and concentrated. Compound **7b** was purified by column chromatography: 1.16 g, 67% yield, mp 45−47 °C; NMR ($CDCl_3$) δ 4.42 (2 H, s, CH_2Br), 5.05 (2 H, s, OCH_2), 6.8−7.7 (9 H). The mixture obtained by adding this compound (1.08 g, 3.90 mmol), methyl 4-hydroxybenzoate (0.593 g, 3.90 mmol), and K_2CO_3 (1.348 g, 9.75 mmol) in 2-butanone (65 mL) was refluxed for 6 h and then filtered. The filtrate was washed with ethyl acetate, and the organic phase was washed with water (2 × 20 mL), dried, and evaporated to give an amber yellow thick oil, which was chromatographed to give **8b** as a white solid: 0.78 g, 57% yield, mp 73−75 °C; NMR ($CDCl_3$) δ 3.90 (3 H, s, OCH_3), 5.07 (4 H, s, OCH_2), 6.8−7.5 (11 H), 8.0 (2 H, m). Under nitrogen, $LiAlH_4$ (3.4 mL, 1 M solution in THF, 3.36 mmol) was dissolved in anhydrous THF (35 mL). Compound **8b** (0.78 g, 2.24 mmol) dissolved in THF (25 mL) was added dropwise at 0 °C, and then the mixture was stirred at reflux for 5.30 h. After addition of ethyl acetate (1 mL) and H_2SO_4 (10%, 1 mL), the solution was filtered and evaporated. Ethyl acetate (30 mL) and water (30 mL) were added to the residue. The organic phase was dried and evaporated to give **9b** as a colorless oil, which solidified slowly: 0.68 g, 95% yield, mp 66−67 °C; NMR ($CDCl_3$) δ 4.62 (2 H, s, CH_2OH), 5.02 (2 H, s, OCH_2), 5.07 (2 H, s, OCH_2), 6.8−7.5 (13 H). Compound **9b** (0.68 g, 2.12 mmol) was treated with tetrabromomethane (1.054 g, 3.18 mmol) and triphenylphosphine (0.384 g, 3.18 mmol) as indicated above, giving the title compound **10b** as a colorless thick oil: 0.54 g, 66% yield; NMR ($CDCl_3$) δ 4.50 (2 H, s, CH_2Br), 5.02 (2 H, s, OCH_2), 5.07 (2 H, s, OCH_2), 6.8−7.5 (13 H).

Synthesis of Enantiomers (+)-3a and (−)-3a. These were prepared following procedure A starting from (*R*)-(+)-**2** and (S)-(−)-**2**, respectively. Compound (*R*)-(+)-**3a** was obtained in 70% yield and had $[\alpha]_D$ = +52 (*c* 1, MeOH). Compound (*S*)-(−)-**3a** was obtained in 53% yield and had $[\alpha]_D$ = −51.7 (*c* 1, MeOH). The optical purity was >95% and was determined by an LIS experiment adding a percentage from 5 to 25% of tris-[3-(heptafluoropropyl)hydroxymethylene-(+)-camphorate)−europium(III)].

Synthesis of Enantiomers (+)-4a and (−)-4a. These were prepared as described above for compounds (+)-**3a** and (−)-**3a**. Enantiomer (+)-**4a** was obtained in 37% yield and had $[\alpha]_D$ = +45.5 (*c* 1, MeOH). Enantiomer (−)-**4a** was obtained in 64% yield and had $[\alpha]_D$ = − 44.9 (*c* 1, MeOH). For both compounds the optical purity, determined by a similar LIS experiment, was >95%.

Partition Coefficients. The log *P* (where *P* is the octanol−water partition coefficient) was obtained as described by Arnoldi and Merlini (1990) using reversed-phase chromatography (Braumann, 1986) by comparison with 13 reference compounds having known log *P* values (Nys and Rekker, 1974). Retention times were determined on a Hewlett-Packard HPLC equipped with a quaternary pump HP-1050 with a Rheodyne injector (20 μL loop) with an UV−visible detector HP-1050 or a Waters Lambda Max model 481. Methanol for HPLC was purchased from Baker, and water for HPLC was produced with a Milli-Q water purification system (Millipore). Analyses were performed on a Merck column LiChrospher 100 RP18 (250 × 4 mm, 5 μm), and the flow rate was 1 mL/min. As the log *P* values of the compounds fall in a large range, it was necessary to use three different eluents [methanol/water, 20:80 (**4h** and **4i**), 70:30 (compound **5**), and 80:20 (all others)] and different groups of standards.

Scheme 1



Molecular Modeling. The molecular models for all compounds were built on a Silicon Graphics IRIS 35, using the program INSIGHT II, 95.0 (Molecular Simulation Inc., San Diego, CA). The initial models were refined by molecular mechanics techniques: the DISCOVER program (Molecular Simulation Inc.) was used to generate low-energy conformations. The electronic charges and the dipole moments were calculated via electrostatic potential in MOPAC 6.0, using AM1 parametrization.

QSAR. The program Cerius[2] 3.0 (Molecular Simulation Inc.) was used to perform the QSAR study.

Biological Assays. The antifungal activity was tested in vitro on *Botrytis cinerea* Pers., *Cercospora beticola* Sacc., *Cercosporella herpotrichoides* Fron., *Fusarium roseum* Link, *Helminthosporium maydis* Nisik. and Miyake, *Helminthosporium teres* Sacc., and *Ustilago maydis* (DC) Corda. The in vivo activity was assayed against cucumber powdery mildew [*Sphaerotheca fuliginea* (Sch. ex Fr.) Poll.] and bean rust [*Uromyces appendiculatus* (Pers.) Link.]. The pathogens were selected in part because they are normally controlled by azoles and in part because they are insensitive. The results were compared with those obtained with tetraconazole.

In Vitro Assays. The activity was determined as inhibition of the radial growth on potato dextrose or Czapek agar Difco, under a series of concentrations (100−0.1 mg L^{-1}). ED_{50} values were calculated for each compound.

Compounds **3a**, **4a**, **(+)-4a**, and **(−)-4a** were also tested on *U. maydis* grown in Coursen and Sisler broth (Carzaniga et al., 1991b). These cultures were used for morphological observations under interference contrast and fluorescence microscopy and for sterol analysis.

In Vivo Assays. Cucumber and bean plants were grown under growth room conditions (T = 22 ± 1 °C, RH = 80 ± 10%) in plastic pots. The tested compounds dissolved in acetone/distilled water (1:4 v/v) containing Tween 20 (0.4 mg mL^{-1}) were sprayed on plant leaves until moist, at concentrations ranging from 100 to 0.001 mg L^{-1}.

In preventive tests, 1 day later cucumber plants were inoculated with a water suspension of conidia of the fungus *S. fuliginea* (1.5 × 10^5 conidia mL^{-1}), and bean plants were inoculated with a uredospore suspension of *U. appendiculatus* (1 × 10^5 spores mL^{-1}). After inoculation, the cucumber plants were placed immediately in a growth room (T = 22 ± 1 °C, RH = 80 ± 10%); the bean plants were placed in the growth room after a period of 24 h in an incubation cabinet at T = 22 ± 2 °C and 100% RH. The biological activity was evaluated 6, 8, and 10 days after inoculation and was expressed as MIC.

In curative tests, compounds were applied 24 h after inoculation

Inhibition of Sterol Biosynthesis. The procedure described by Gozzo et al. (1995) was applied to compounds **4a**, **(+)-4a**, and **(−)-4a**.

Light Microscopy. Light microscopy examinations were performed on *U. maydis* sporidia as described by Carzaniga et al. (1991b).

Possible Formation of Compound 5 in Broth Culture. Broth cultures of *U. maydis* (100 mL) were prepared as described for sterol analysis. Compounds **3c** and **4b** were added at 100, 5, and 1 mg/mL concentrations. After 18 h of incubation, the cultures were extracted with ethyl acetate (3 × 50 mL). The combined organic phases were dried, concentrated, and analyzed by GC and GC-MS. Analyses were conducted on a DB-5 (J&W Scientific) column, 30 m × 0.25 mm, film 0.25 μm, using the following program: 180 °C for 3 min, 5 °C/min to 240 °C, held for 10 min. In these conditions compound **5** had t_r = 7.50 min, compound **4b**, 10.25 min, and compound **3c**, 23.65 min. No peak corresponding to **5** could be detected in the extracts.

RESULTS AND DISCUSSION

Chemistry. With only a few exceptions, the ethers of structures **3** and **4** were prepared from alcohol **2** and the suitable benzyl or allyl halide as indicated in procedure A (Table 1). After many trials in different conditions, phase transfer catalysis (McKillop et al., 1974; Schubert et al., 1983) was found to be the best way to attain this nucleophilic substitution; nevertheless, reactions with alkyl halides failed.

Ether **4g** was obtained by catalytic hydrogenation of the cinnamyl derivative **4f** and nitrile **4h** by a Michael reaction of alcohol **2** with acrylonitrile in the presence of Triton-B (Bruson, 1949). Alcohol **4i** was synthesized from alkene **4a** in a two-step procedure, first with 9-borabicyclononane and then with hydrogen peroxide (Burgess and Ohlmeyer, 1991).

The two alkyl bromides **10a,b** were prepared by the sequence of reactions outlined in Scheme 1. The halides **7a,b** were treated with methyl 4-hydroxybenzoate and K_2CO_3 as a base in 2-butanone. The esters **8a,b** were reduced with lithium aluminum hydride to the alcohols **9a,b**, which were converted into the bromoderivatives **10a,b** by treatment with CBr_4 and triphenylphosphine.

Compounds **3a** and **4a** were selected for the preparation of pure enantiomers. They were synthesized by alkylation, as described above, of the alcohols *R*-(+)-**2** and *S*-(−)-**2,** which had been prepared by enantioselective hydrolysis of the racemic acetate by pig pancreatic lipase following the procedure of Bianchi et al. (1991). The optical purity of each enantiomer was >95% as ascertained by LIS experiments.

Although compound **5** was repeatedly obtained as a byproduct when alcohol **2** was reacted in different conditions, it was not possible to obtain a sufficiently pure sample in this way. A five-step procedure was therefore applied. 2,4-Dichloroacetophenone was reacted with methylmagnesium bromide to give the tertiary alcohol **11**, which was acetylated to facilitate the

Table 2. In Vitro Fungicidal Activity Expressed as 50% Effective Concentration (EC_{50}, mg L^{-1})

compd	*B. cinerea*	*C. beticola*	*C. herpot*	*F. roseum*	*H. maydis*	*H. teres*	*U. maydis*
3a	1.6	0.20	0.35	5.0	0.18	0.70	0.14
(+)-3a	1.3	0.18	0.60	13	0.25	0.35	0.09
(−)-3a	3.0	0.16	0.41	18	0.25	0.50	<0.10
3b	2.8	0.09	0.18	>200	0.15	0.75	0.08
3c	1.0	0.10	0.35	10	0.15	0.45	0.07
3d	3.8	0.28	1.2	6.5	0.40	0.80	0.10
3e	1.0	0.18	0.65	12	0.28	0.35	0.09
3f	2.8	0.23	1.0	130	0.15	0.25	0.18
3g	2.8	0.18	0.50	>200	0.18	0.55	0.03
3h	8.0	0.14	5.0	>200	150	0.85	0.16
3i	3.5	0.20	0.50	>200	0.15	0.22	0.14
3l	25	0.90	1.8	>100	nd[a]	1.3	0.20
3m	100	0.18	0.16	>100	nd	0.20	0.20
3n	2.0	0.20	1.20	1.5	0.20	0.70	0.10
4a	1.5	0.21	0.29	8.5	0.16	1.4	0.22
(+)-4a	1.50	<0.10	0.28	10	0.18	1.50	0.09
(−)-4a	3.0	0.25	0.40	8.5	0.20	0.80	0.22
4b	1.2	0.15	0.50	13	0.10	0.75	0.25
4c	0.90	0.18	1.0	40	0.13	0.50	0.22
4d	0.80	0.23	0.50	12	nd	2.0	0.50
4e	3.6	0.30	0.50	25	nd	1.4	0.50
4f	3.0	0.20	1.3	40	0.18	0.47	0.10
4g	2.3	0.18	2.3	18	0.19	1.30	0.15
4h	18	4.0	20	32	8.5	32	4.0
4i	70	1.70	2.5	75	1.3	8.0	1.0
5	4.5	0.60	0.45	2.9	0.40	0.90	0.80
tetraconazole	1.2	0.12	0.45	4.0	<0.10	0.40	0.25

[a] nd, not determined.

thermal elimination to give the 2-phenylprop-1-ene **13** (Seymour and Wolfstirn, 1948). This compound was brominated with *N*-bromosuccinimide (Luteri and Ford, 1977) to give a 57:43 mixture (by NMR) of 3-bromo-2-(2,4-dichlorophenyl)prop-1-ene, **15**, and 1-bromo-2-(2,4-dichlorophenyl)prop-1-ene, **14**. Without any separation, the mixture was treated with 1,2,4-triazole in triethylamine: only compound **15** reacted in these conditions, giving compound **5** easily separable from unreacted **14** by column chromatography.

In Vitro Assays. The in vitro fungitoxicity (expressed as ED_{50}) is reported in Table 2 in comparison with that of tetraconazole. The compounds tested exhibited a broad spectrum of activity, very low efficacy being shown only against *F. roseum* except for a few cases. Very good fungitoxicity was observed with the majority of the compounds, and compounds **3a**, **3b**, **3c**, **4a**, **4b**, and **4c** showed ED_{50} values lower than or equivalent to that of tetraconazole on at least six of the tested fungi. Summarizing, compounds **3d**, **3e**, **3f**, **3g**, **3i**, **3m**, **3n**, **4f**, and **4g** shared a high activity on *H. maydis*, *H. teres*, and *U. maydis*; **3d** was very active also on *F. roseum*, **3e** on *B. cinerea*, and **3m** on *C. herpotrichoides*. Compounds **3h**, **3l**, **4d**, and **4l** were generally less active, but **3h** and **3l** maintained high activity on *U. maydis* and as did **4d** on *B. cinerea*. Compound **5** was characterized by a very high activity on *F. roseum*, and compounds **4h** and **4i** exhibited a very low activity on all of the fungi.

The compounds of class **3** are benzyl ethers bearing substituents with different physicochemical characteristics. The most active compounds, **3a**, **3b**, and **3c**, bear rather small and polar substituents such as H (**3a**), NO_2 (**3b**), or OCH_3 (**3c**), whereas two chlorine atoms (**3e**), a *tert*-butyl group (**3d**), a naphthalene group (**3f**), a phenyl group (**3g**), and longer or larger chains (see **3m**) reduce the efficacy (this was even more evident with the ED_{90} values; data not shown). A linear shape with no branching seems to be important for activity (compare **3h** with **3g** and **3i** and compound **3l** with **3m)**. The substitution of the isosteric thienyl group for the phenyl group preserves the activity (compare **3n** and **3a**).

Class **4**, in general, is less effective than class **3**: only the first three compounds, **4a**, **4b**, and **4c**, which contain an allyl substituent instead of a benzyl one, have an activity similar to that of some elements of class **3**. However, bulky substituents on the allyl group, such as a chlorine atom (**4d**) or an aromatic ring (**4f**), have a negative effect. The compounds that contain a small polar saturated substituent instead of an allyl group, such as **4h** or **4i**, although not completely ineffective, are the worst members of the series.

Two compounds (**3a** and **4a**) were prepared as single enantiomers and assayed. On the whole *R*-(+)-**3a** and *S*-(−)-**3a** were found to be equivalent and not much different from the racemate; on the contrary *R*-(+)-**4a** was slightly more active than *S*-(−)-**4a**.

Not being a substituted ether, compound **5** must be discussed separately. As already indicated in the Introduction, it was synthesized to verify the hypothesis that it could be a common fungitoxic metabolite of compounds **3** and **4** in vivo. It was generally not very active, but, surprisingly, better than the standard tetraconazole on *F. roseum*.

To establish whether **5** could be formed in vivo in broth cultures, compounds **3c** and **4b** were incubated for 18 h in the presence of *U. maydis* sporidia, and, at the end of this period, the cultures were extracted with ethyl acetate and the presence of metabolites was checked by GC-MS. As no peak of compound **5** was detected, the hypothesis that it is a common active metabolite was excluded.

QSAR Studies. To put in evidence which are the most relevant physicochemical features affecting in vitro fungitoxicity, it was decided to perform a QSAR study. However, two compounds (**3l** and **3m**) had to be discarded because they are very flexible, a fact that makes

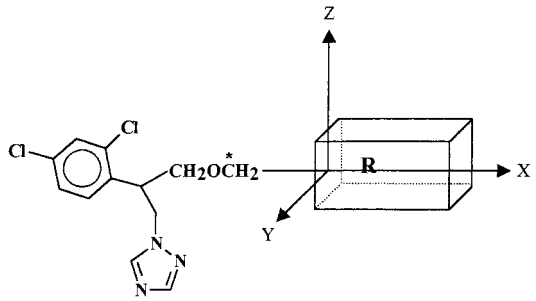


Figure 2. Definition of some of the parameters of Table 3. Geometrical descriptors: $|X|_{max} = X1$; $|Y|_{max} = Y1$; $|Y|_{min} = Y2$; $|Z|_{max} = Z1$; $|Z|_{min} = Z2$. Charge: * indicates the carbon used for the charge.

it very difficult to choose the best conformation to be used for the calculation of the descriptors.

It is well-known that the activity depends on the size, shape, and functional groups of the whole molecule, but, as this work concerns a set of derivatives of a parent compound in which the moiety containing the 2,4-dichlorophenyl and 1,2,4-triazolyl groups remains constant, some of the parameters refer only to the variable part of the molecule (R in the general formula).

Due to the difficulty of finding all the descriptors in the file compiled by Hansch and Leo (1979), parameters similar to the well-known STERIMOL ones (Verloop et al., 1976), but not taking in account the van der Waals radii, were obtained from the refined structures. *X* corresponds to the *L* parameter of STERIMOL; *Y*1, *Y*2, *Z*1, and *Z*2 are analogous to *B*1, *B*2, *B*3, and *B*4, respectively (Figure 2).

van der Waals volumes (VdW) were calculated on the minimum energy conformation of the whole molecule. The electronic parameters were calculated via electrostatic potential in MOPAC 6.0; the parameter "charge" represents the nonscaled charge of the first C of substituent R (indicated with * in the general formula of Figure 2), whereas the dipole moments (μ) are referred to the whole molecule. An experimental procedure based on HPLC retention times was utilized to obtain the log *P* values (Arnoldi and Merlini, 1990).

The molecular descriptors (Table 3) were correlated with the biological activities expressed as log 1/*C* (where *C* is the EC_{50} in mol L^{-1}) on the fungi *U. maydis*, *C. beticola*, *C. herpotrichoides*, *H. teres*, and *B. cinerea*. A simple stepwise regression method was applied using the program Cerius^2 3.0 (Molecular Simulation Inc.), which gave the following equations:

B. cinerea

$$\log 1/C = 3.850 + 0.735(\log P) - 0.082 (\log P)^2 - 0.162(Z2)$$

$$(r^2 = 0.822;\ XVr^2 = 0.640;\ PRESS = 1.526)$$

C. beticola

$$\log 1/C = 4.459 + 0.664(\log P) - 0.082(\log P)^2 + 0.150\mu$$

$$(r^2 = 0.933;\ XVr^2 = 0.869;\ PRESS = 0.413)$$

C. herpotrichoides

$$\log 1/C = 3.895 + 0.841(\log P) - 0.130(\log P)^2 + 0.236\mu - 0.178(Z2)$$

$$(r^2 = 0.831;\ XVr^2 = 0.521;\ PRESS = 2.064)$$

H. teres

$$\log 1/C = 3.714 + 0.330(\log P) + 0.194\mu$$

$$(r^2 = 0.829;\ XVr^2 = 0.677;\ PRESS = 1.724)$$

U. maydis

$$\log 1/C = 4.447 + 0.280(\log P) + 0.237\mu$$

$$(r^2 = 0.814;\ XVr^2 = 0.730;\ PRESS = 1.249)$$

For each equation important indices of goodness of fit are given: correlation coefficient, r^2; cross-validation correlation coefficient, XVr^2; and predicted sum of squares, PRESS. During the procedure of cross-validation, each molecule is left out, in turn, and the r^2 is computed using the predicted values of the missing molecule. The cross-validation correlation coefficient XVr^2 is a measure of the predictive power of the equation. The predicted sum of squares is the sum, over all compounds, of the squared differences between the actual and predicted values for the independent variable. The best correlation was obtained for *C. beticola*.

In all equations, lipophilicity is a major positive parameter affecting the activity; the dependence is linear for *U. maydis* and *H. teres* and parabolic for *C.*

Table 3. Physicochemical Parameters for the Stepwise Regression

compd	VdW	*X*	*Y*1	*Y*2	*Z*1	*Z*2	log *P*	log^2 *P*	charge	μ	*U. maydis*	*C. beticola*	*C. herpot.*	*H. teres*	*B. cinerea*
3a	269.14	3.875	2.152	2.153	0.017	0.725	3.83	14.67	0.0075	3.39	6.41	6.26	6.01	5.71	5.35
3b	286.71	4.766	2.163	2.164	0.818	0.816	3.72	13.84	0.0109	5.75	6.71	6.65	6.35	5.73	5.16
3c	290.57	6.139	2.158	2.149	0.897	0.897	3.85	14.82	0.0667	4.22	6.75	6.59	6.05	5.94	5.59
3d	327.35	6.210	2.161	2.159	2.199	2.196	4.84	23.43	0.0324	3.41	6.50	6.05	5.42	5.60	4.92
3e	299.21	4.558	2.153	2.765	0.017	0.752	4.71	22.18	−0.0777	3.98	6.68	6.38	5.82	6.09	5.63
3f	305.29	5.966	3.392	2.159	0.025	0.706	4.48	20.07	0.0351	3.52	6.36	6.25	5.61	6.22	5.17
3g	329.75	8.139	2.169	2.175	0.999	0.997	4.29	18.40	0.0239	3.46	7.16	6.39	5.94	5.90	5.19
3h	336.37	5.631	2.151	5.384	0.037	4.442	4.58	20.98	−0.0396	3.74	6.45	6.51	4.96	5.73	4.75
3i	351.32	10.199	2.157	2.149	2.172	2.133	4.46	19.89	0.0675	4.32	6.52	6.37	5.97	6.33	5.13
3n	264.60	3.432	1.198	2.322	0.030	0.733	4.42	19.54	0.1975	3.43	6.57	6.26	5.49	5.72	5.26
4a	235.82	1.778	2.143	0.916	0.535	0.120	3.49	12.18	0.1296	3.05	6.15	6.17	6.03	5.35	5.32
4b	244.78	1.759	2.128	2.137	0.606	1.374	3.72	13.84	−0.2042	3.10	6.11	6.34	5.81	5.64	5.43
4c	259.36	2.730	3.365	1.041	1.268	0.539	3.91	15.29	0.1561	3.12	6.19	6.27	5.53	5.83	5.58
4d	246.73	1.773	2.141	1.461	0.149	0.541	3.70	13.69	0.1831	1.81	5.84	6.18	5.84	5.24	5.63
4e	225.96	2.100	0.965	0.802	0.612	0.052	2.93	8.580	0.0729	2.97	5.79	6.01	5.79	5.34	4.93
4f	292.33	6.066	2.967	0.971	1.514	1.781	4.21	17.72	0.0567	3.15	6.59	6.29	5.47	5.92	5.11
4g	296.84	6.099	1.866	0.695	2.140	2.161	4.77	22.75	−0.0121	2.89	6.41	6.33	5.23	5.48	5.23
4h	235.52	1.173	2.249	0.619	0.913	0.890	0.36	0.130	0.0628	2.34	4.92	4.92	4.22	4.02	4.26
4i	242.51	2.511	1.943	0.684	0.914	0.888	0.32	0.102	−0.1048	3.23	5.51	5.28	5.11	4.61	3.67

Table 4. In Vivo Fungicidal Activity Expressed as MIC (mg L^{-1})

compd	U/P[a] direct protectant	U/P curative	S/C[b] direct protectant	S/C curative
3a	30	35	50	
(+)-3a	50		100	
(−)-3a	50		80	
3b	43		10	
3c	30	12	35	
3d	25		10	10
3e	30		10	
3f	120		50	
3g	28		10	6
3h	50		12	
3i	35		10	
3l	140			
3m	>100			
3n	200		100	
4a	200	28	30	
(+)-4a	25		90	
(−)-4a	100		100	
4b	150		50	
4c	160		100	
4d	>100			
4e	>100			
4f	140		10	
4g	200		100	
4h	250		60	
4i	>200		>100	
5	150		8	1.0
tetraconazole	25	25	0.05	0.9

[a] U/P *Uromyces appendiculatus/Phaseolus vulgaris*. [b] S/C *Sphaerotheca fuliginea/Cucumis sativus*.

beticola, *C. herpotricoides*, and *B. cinerea*. This means that molecules with an intermediate lipophilicity (practically when the log *P* is in the range 3.5−4.2) are the best. The second relevant parameter is μ, which has a positive effect in all cases but on *B. cinerea*.

C. herpotricoides and *B. cinerea* are sensitive also to *Z*2, the parameter that, corresponding roughly to STERIMOL *B*4 and having a negative coefficient, indicates that wide substituents are not acceptable and that linear and narrow ones are more suitable. The van der Waals radii, the charge of the first C of the substituent, and the length of the substituent do not affect significantly the biological activity.

The data were treated also with a genetic algorithm, which gave the same results, perhaps because the descriptors are relatively few.

In Vivo Assays. The protective activity of the new compounds, assayed against bean rust and cucumber powdery mildew, is listed in Table 4. Many compounds controlled both diseases. The most active ones belonged to class **3**. The comparison with tetraconazole showed that compounds **3a**, **3c**, **3d**, **3e**, and **3g** controlled bean rust as much as the standard, whereas the activity against cucumber powdery mildew was always remarkably lower.

Curative tests were carried out only for a few compounds (see Table 4). Compounds **3a**, **3c**, and **4a** exhibited an excellent curative activity against bean rust, compound **3a** being superior to tetraconazole. Compound **5** was the only one that showed the same activity as tetraconazole in curative tests against cucumber powdery mildew.

As in in vitro assays, the two enantiomers of **3a** and **4a** had very similar behaviors in vivo, only *R*-(+)-**4a** appeared to be clearly more fungitoxic than *S*-(−)-**4a** on bean rust.

Light Microscopy. Growth and morphological abnormalities have been detected in both budding and filamentous fungi under the action of azole fungicides (Ragsdale and Sisler, 1973; Buchenauer, 1975, 1976; Weete at al., 1983; Kerkenaar and Barug, 1984; Roberson et al., 1989).

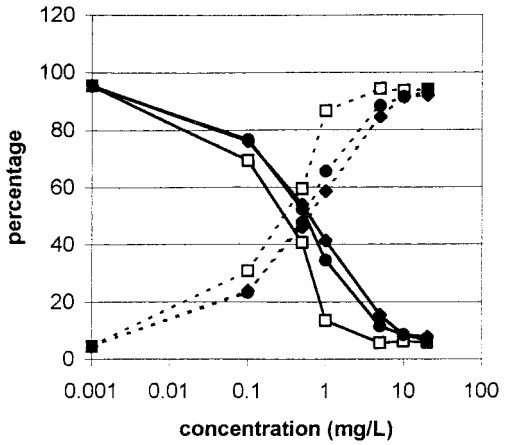


Figure 3. Sterol composition of *U. maydis* sporidia (ATCC 14826) after 18 h of treatment with increasing concentrations of racemic **4a**, **(+)-4a**, and **(−)-4a** added to actively growing cultures: (—) 14α-demethylsterols; (- - -) 14α-methylsterols; (●) racemic **4a**; (□) **(+)-4a**; (◆) **(−)-4a**.

The observations carried out on sporidia of *U. maydis*, treated with some of the most active compounds, by Normasky optics and fluorescence microscopy, showed morphological changes similar to those induced by tetraconazole (Carzaniga et al., 1991b). Concentrations from 0.25 to 50 mg mL^{-1} affected cell division; after 18 h of treatment, sporidia appeared to be swollen, multicellular, and branched. An intensive fluorescence was localized in the septa and as patches over the cell wall indicating areas of irregular chitin deposition.

This feature has been described by a number of authors as a typical effect of ergosterol inhibitors (Kerkenaar and Barug, 1984; Sancholle et al., 1988).

Far more extensive changes were induced when sporidia were exposed to concentrations ≥100 μg mL^{-1}. Cells showed an immediate irreversible collapse, and depressions were evident on their surface. These morphological damages suggest that a different mechanism is probably operating at high concentrations, as already proposed by other authors (Dahmen at al., 1988; Lyr and Müller, 1990).

Sterol Analysis. The presence of the 1,2,4-triazole ring and the structural similarities with tetraconazole (Carzaniga et al., 1991a) suggested that the activity of these compounds was due to an inhibition of sterol biosynthesis at level of 14α-demethylase. To confirm this hypothesis, broth cultures of *U. maydis* (ATCC 14826) were incubated for 18 h with increasing concentrations of **4a** or its enantiomers, and the sterols were extracted and analyzed.

In the control, demethylsterols were mainly represented by ergosterol (61%) and ergosta-5,7-dien-3-ol (26.6%) and other minor demethylsterols such as ergosta-5,7,22,24(28)-tetraenol, ergosta-5,7,24(28)-trienol, ergosta-7,22-dienol, and ergosta-7-enol (totally 7.7%). The 14α-methylsterols include eburicol (3.4%), the normal substrate of 14α-demethylase, minor amounts of obtusifoliol (0.7%), and 14α-methylfecosterol (0.4%). All of these compounds had been fully characterized in a previous work (Carzaniga et al., 1991a).

The change in the sterol composition induced by the antifungal treatments is shown in Figure 3 (summations

of 14α-demethylsterols and 14α-methylsterols are reported). Under the action of the three tested compounds, ergosterol and ergosta-5,7-dien-3-ol were drastically depleted, whereas eburicol and obtusifoliol, which bear one and two methyl groups in position 4, respectively, represented the major part of the sterols accumulated. For example, with 20 mg mL^{-1} of the racemate **4a**, eburicol reached 61.1%, obtusifoliol 20.6%, and 14α-methylfecosterol 8.8%, whereas ergosterol was reduced to 5.1%. This is the standard behavior observed when *U. maydis* is treated with sterol biosynthesis inhibitors that have 14α-demethylase as the principal target enzyme (Berg, 1986; Shephard et al., 1986; Waterfield and Sisler, 1987).

It is interesting to observe that at low doses of the inhibitors, one of the last precursors of ergosterol, ergosta-5,7-dien-3-ol, slightly accumulated, whereas at high doses its amount decreased until it became <1% (data not shown). Similar observations have been made by Sisler and Ragsdale (1977), Takano and Kato (1987), and Gozzo et al. (1995) after treatments of *U. maydis* with triarimol, diniconazole, or tetraconazole, respectively. The introduction of the double bond in position 22,23 that, in the strain under investigation, has as principal substrate ergosta-5,7-dien-3-ol, is another step depending on a cytochrome P450 (Hata et al., 1981). Of course, this effect is detectable only at low doses, at which the inhibition of 14α-demethylase is not very pronounced.

Figure 3 indicates clearly that 14α-demethylsterol decreased proportionally with the increase of the fungicide dose. The I_{50} values (concentration which reduces to 50% the amount of 14α-demethylsterols) were 0.54 mg L^{-1} for (±)-**4a**, 0.28 mg L^{-1} for the (+)-isomer, and 0.62 mg L^{-1} for the (−)-isomer. These values match very well the EC_{50} values [0.39 mg L^{-1} for (±)-**4a**, 0.34 mg L^{-1} for the (+)-isomer, and 0.50 mg L^{-1} for the (−)-isomer] obtained on growth inhibition in the same broth cultures. Nevertheless, it must be observed that the differences between the racemate and the single enantiomers are relatively small with respect to tetraconazole (Gozzo et al., 1991, 1995). Carelli et al. (1992) have demonstrated that the relative activity of the enantiomers of tetraconazole can be explained by their different relative affinities with the target enzyme.

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