Quantitative Structure Activity Relationships of Fungicidally Active Triazoles: Analogs and Stereoisomers of Propiconazole and Etaconazole

E. Ebert[+], W. Eckhardt[+], K. Jäkel*, P. Moser*, D. Sozzi[+], and C. Vogel[+]

Agricultural Division and Physics Department CIBA-GEIGY Limited, 4002 Basel, Switzerland

Z. Naturforsch. **44c**, 85–96 (1989); received September 8, 1988

Propiconazole, Etaconazole, Triazoles, Fungicides, QSAR

The preparation of the four stereoisomers of propiconazole (TILT®) is described. Their inhibition of the 14α-C-demethylation of the sterol nucleus is examined and compared with the inhibition by the four stereoisomers of etaconazole (SONAX®). The quantitative structure-activity relationships (QSAR) of substituted 1,3-dioxolane-2-yl-methyltriazoles and 1,3-dioxane-2-yl-methyltriazoles on *in vivo* fungicidal activity are investigated.

Introduction

In recent years it has been shown, that several chemicals with fungicidal activity are inhibitors of sterol biosynthesis [1, 6, 20]. One important group of such fungicides are derivatives of 1,2,4-triazoles like triadimefon, diclobutrazole, flutriafol, flutrimafol, etaconazole, propiconazole or penconazole (Fig. 1), which are active against powdery mildews like *Erysiphe graminis* DC., rusts like *Puccinia graminis* Pers. and leaf spot diseases like *Cercospora arachidicola* Hori. It has been shown, that these triazole fungicides block ergosterol biosynthesis in fungi by inhibiting sterol 14α-demethylation, a process mediated by a cytochrome P-450 enzyme [2–6].

In this study we have investigated quantitative structure activity relationships (QSAR) of a series of 2-phenyl-1,3-dioxolan-2-yl-methyl-1-H-1,2,4-triazoles and 2-phenyl-1,3-dioxane-2-yl-methyl-1H-1,2,4-triazoles I (Fig. 2) comprising the prominent compounds etaconazole (SONAX®) and propiconazole (TILT®) (Fig. 1). These two compounds were first synthesized by Janssen Pharmaceutical Research Laboratories in Belgium [7, 8] and were later developed as plant fungicides by the Agrochemical Division of Ciba-Geigy.

This first report on the QSAR's of these chemicals will focus on *in vivo* fungicide tests performed in the greenhouse. Results of *in vitro* enzyme inhibition tests are reported and discussed only for the four stereoisomers of etaconazole and propiconazole.

Reprint requests to Dr. W. Eckhardt.

[+] Agricultural Division.

* Physics Department, Central Function Research.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/89/0100–0085 $ 01.30/0

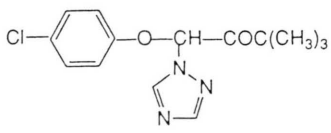
Materials and Methods

Chemical compounds

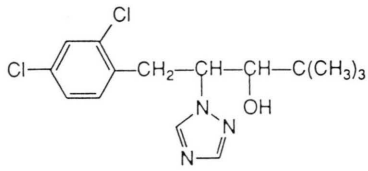
The synthesis of the isomeric mixtures of the title compounds has been described by G. van Reet *et al.* [7]. The synthesis of the four isomers of etaconazole (SONAX®) has been published by C. Vogel *et al.* [9]. Here we want to describe the synthesis of the four isomers of propiconazole (TILT®) (Fig. 3).

S- and *R*-α-Hydroxyvaleric acid

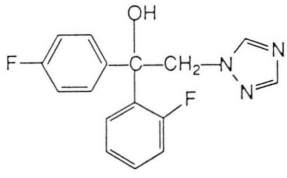
The *S*- or *R*-α-hydroxyvaleric acids were synthesized as published by Winitz *et al.* [10] by deamination of the optically pure *S*- or *R*-α-aminovaleric acids with nitrous acid. They were purified by crystallization of their salts with *R*(+)- or *S*-(-)-1-phenylethylamine in acetonitrile. The pure salts have melting points of 96–99 °C. [S-acid/S-amine-salt: $[\alpha]_D^{20} = -20 \pm 1°$ ($c = 3.16\%$ in methanol); *R*-acid/*R*-amine-salt: $[\alpha]_D^{20} = +20 \pm 1°$ ($c = 2.75\%$ in methanol)]. The free acids were obtained by treating the salts with conc. hydrochloric acid in dioxane. 96.0 g (0.4 mol) *S*-acid/*S*-amine-salt in 1500 ml dioxane were treated with 40 ml conc. hydrochloric acid. The colourless, clear solution was evaporated under reduced pressure at 50 °C, the viscous residue was dissolved in 500 ml dioxane. By adding 2500 ml diethyl ether the hydrochloride of the amine was precipitated under stirring. The fine sediment was filtered off, washed with diethyl ether and the filtrate was evaporated at 50 °C/1600 Pa. The residue was a pale



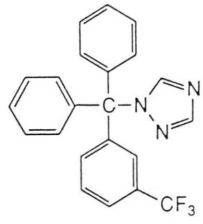
Triadimefon
Bayleton®
Bayer AG



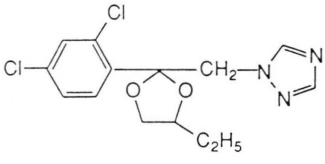
Dichlobutrazole
Vigil®
ICI



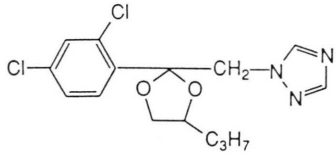
Flutriafen
Impact®
ICI



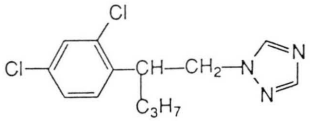
Fluotrimazole
Persulon®
Bayer AG



Etaconazole
Sonax®
Ciba-Geigy AG

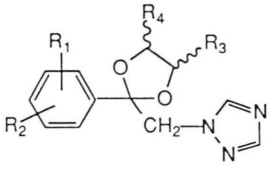


Propiconazole
Tilt®
Ciba-Geigy AG



Penconazole
Topas®
Ciba-Geigy AG

Fig. 1. Structures of some inhibitors of C-14-demethylation in ergosterol biosynthesis.



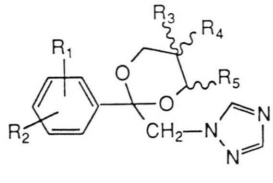


Fig. 2. General formula of the triazoles used in this study.

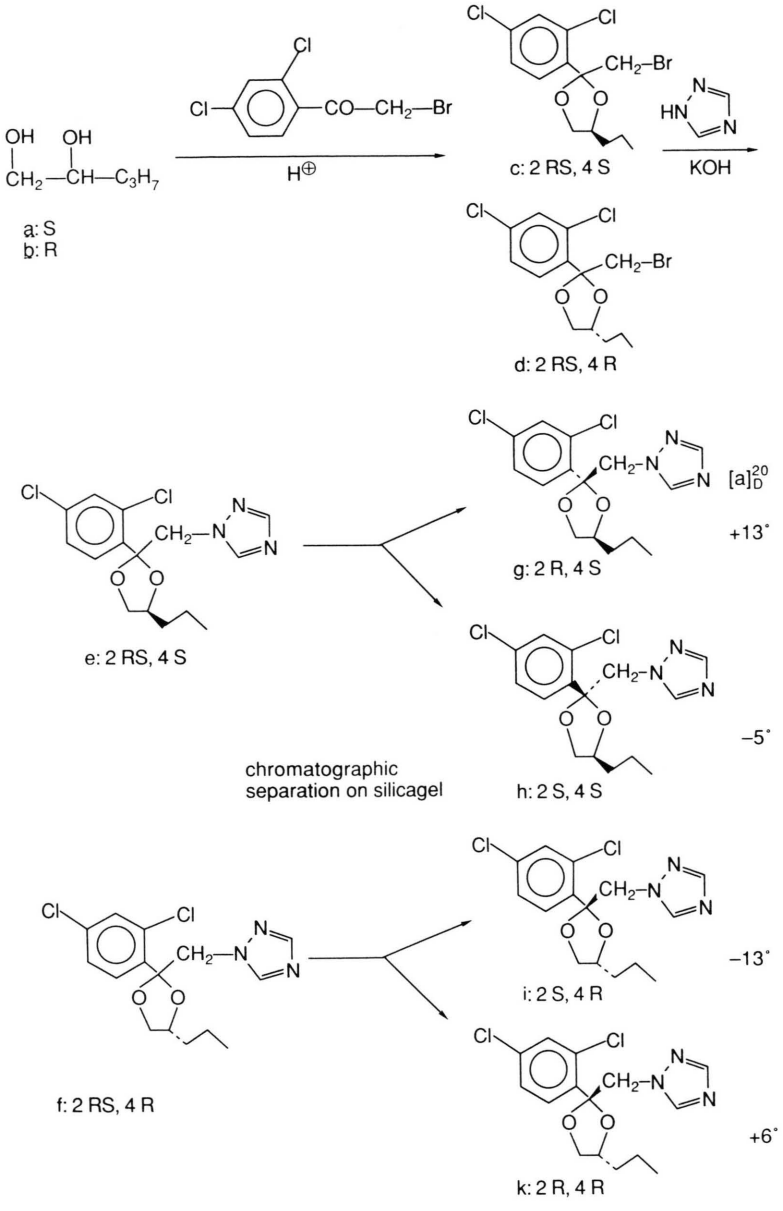


Fig. 3. Synthesis scheme of the four isomers of propiconazole.

yellow oil of *S*-α-hydroxyvaleric acid (61 g containing about 20% dioxane) which was used for further synthesis without other purification.

S- and *R*-1,2-Pentanediols **a** and **b**

To the solution of 75.5 g (0.52 mol) 80% pure *R*-α-hydroxyvaleric acid dissolved in 750 ml tetrahydrofuran, 80 ml (0.8 mol) borane-dimethylsulfide-complex was added dropwise during one hour at 55–65 °C. Then the mixture was stirred for 4 h at the boiling point, cooled to room temperature with ice and mixed with 160 ml methyl alcohol. When the hydrogen formation had ended, 100 ml 0.1 N hydrochloric acid were added. The mixture was stirred for another 0.5 h, and the solvent was evaporated at reduced pressure. The partly crystalline residue was dispersed in 2000 ml diethyl ether, the boric acid was

filtered off and the solvent evaporated. The residue was dissolved in 800 ml water, the pH brought to 13 by adding barium hydroxide and the slurry filtered through silica gel. The filtrate was evaporated under reduced pressure at 60 °C to a volume of 300 ml, diluted with 1000 ml acetone and the barium borate filtered off. The solid was washed with acetone and the solvent evaporated. The residue was dissolved in 1500 ml diethyl ether, the solution dried with sodium sulfate, filtered and evaporated. The distillation of the residue gave 42.4 g (78%) *R*-1,2-pentanediol **a** as colourless oil, bp.: 105–108 °C/2100 Pa., $[\alpha]_D^{20} = +15 \pm 1°$ ($c = 3.879\%$ in methanol).

The *S*-1,2-pentanediol **b** was synthesized in the same manner with *S*-α-hydroxyvaleric acid as starting material. Yield: 76%, bp. 106–108 °C/2100 Pa., $[\alpha]_D^{20} = -15 \pm 1°$ ($c = 3.03\%$ in methanol).

2*RS*,4*S*-2-(2,4-Dichlorophenyl)-2-bromomethyl-4-*n*-propyl-1,3-dioxolane **c**

The solution of 107.2 g (0.4 mol) 2′,4′-dichloro-2-bromoacetophenone, 47.5 g (0.46 mol) *S*-1,2-pentanediol **a** and 3.5 g *p*-toluenesulfonic acid in 500 ml toluene was boiled in a separator for 16 h. After cooling, 1000 ml diethyl ether was added, the solution was washed with water, then dried with sodium sulfate, filtered and evaporated at reduced pressure. Distillation of the residue gave 126.2 g (89%) of the dioxolane **c** as a pale yellow oil with bp. 125–129 °C/0.1 Pa.

2*RS*,4*R*-2-(2,4-Dichlorophenyl)-2-bromomethyl-2-*n*-propyl-1,3-dioxolane **d**

The synthesis was carried out analogous to the synthesis of dioxolane **c** with *R*-1,2-pentanediol **b** as starting material. Yield: 70.6%, bp.: 128–132 °C/0.1 Pa.

2*RS*,4*S*-1-(2-(2,4-Dichlorophenyl)-4-*n*-propyl-1,3-dioxolane-2-yl-methyl)-1-H-1,2,4-triazole **e**

To a solution of 41.4 g (0.6 mol) 1,2,4-triazole in 350 ml DMSO 33 g (0.5 mol) 85% pure potassium hydroxide were added and stirred at 45 °C until the mixture was a clear colourless solution. Then 126 g (0.356 mol) bromomethyldioxolane **c** were added in 50 ml DMSO. The mixture was stirred at 140 °C during 17 h. The dark brown solution was cooled and 1500 ml ice-water and 2000 ml diethyl ether were added. The water phase was separated and the organic solution was washed with water, dried with sodium sulfate, filtered and evaporated. The mixture of the diastereoisomers **e** was distilled at 155–160 °C/0.1 Pa. and gave a yellow high viscous oil. Yield: 89.3 g (73%).

2*RS*,4*R*-1-[2-(2,4-Dichlorophenyl)-4-*n*-propyl-1,3-dioxolane-2-ylmethyl]-1-H-1,2,4-triazole **f**

The synthesis was analogous to that described for compound **e**. 100.0 g of the bromomethyl-dioxolane **d** gave 58.4 g (61%) of the diastereomeric triazoles **f** as a yellow high viscous oil with bp. 156–160 °C/0.1 Pa.

Separation of the mixtures of the diastereoisomers **e** and **f**

58 g of the mixture of the diastereoisomers **f** were chromatographed on a silica gel column filled with 2200 g silica gel 60 Merck 0.063–0.2 mm mesh and ethyl acetate/hexane = 1:1 as solvent.

2*S*,4*R*-Isomer **i**

30.2 g yellow, viscous oil; $[\alpha]_D^{20} = -13 \pm 1°$ ($c = 3.74\%$ in methanol).

2*R*,4*R*-Isomer **k**

18.7 g yellow, viscous oil; $[\alpha]_D^{20} = +6 \pm 1°$ ($c = 2.73\%$ in methanol).

The mixture of the diastereoisomers **e** was separated on silica gel in the same manner. Yield: 38.3 g pure *"cis"*-compound (2*R*,4*S*) and 28.4 g pure *"trans"*-compound (2*S*,4*S*).

2*R*,4*S*-Isomer **g**

38.3 g yellow, viscous oil; $[\alpha]_D^{20} = +13 \pm 1°$ ($c = 4.14\%$ in methanol).

2*S*,4*S*-Isomer **h**

28.4 g yellow, viscous oil; $[\alpha]_D^{20} = -5 \pm 1°$ ($c = 3.17\%$ in methanol).

Biological activities

In vitro tests with *Ustilago maydis*

Sporidia of *Ustilago maydis* (DC) Cda (CBS 132.08) were cultivated in a nutrient medium and treated with the fungicide as described by Buchenauer *et al.* [11]. Twenty-four hours after

treatment the total lipids were extracted from the washed and lyophilized sporidia with chloroform/methanol (2:1 v/v) and analyzed by gas chromatography following the procedure described by Buchenauer [2]. Cholesterol and ergosterol were used as standards in order to quantify the amount of the various sterol intermediates. The log I_{50} values reported in Table I are averages of three tests with three replications each.

In vivo tests using wheat infected with *Puccinia graminis*

Wheat plants (*Triticum aestivum* L.) were grown in a greenhouse (temperature: 22 °C, relative humidity: 60% RH) using 12 cm-diameter pots filled with standard soil. In order to study the *residual protective activity* of the fungicide, wheat plants were treated 6 days after sowing with a spray mixture prepared from a wettable powder formulation of the active ingredient (concentration 0.006 g/100 ml). After 24 h the treated plants were infected with a uredospore suspension of the fungus. The infected plants were incubated for 48 h at 95–100% relative humidity and about 20 °C and then kept in a greenhouse at about 22 °C. Rust pustule development was evaluated 12 days after infection.

In order to study the *systemic activity* of the fungicides the soil was treated 5 days after sowing with a spray mixture prepared from a wettable powder formulation of the active ingredient (0.06 g/100 ml of soil). After 48 h the treated plants were infected with a uredospore suspension of the fungus. The plants were then incubated for 48 h at 95–100% relative humidity and about 20 °C and kept in a greenhouse at about 22 °C. Rust pustule development was evaluated 12 days after infection.

In vivo tests using groundnut plants infected with *Cercospora arachidicola*

Groundnut (*Arachis hypogaea* L.) plants were cultivated as described for wheat. Plants 10–15 cm in height were sprayed with a spray mixture prepared from a wettable powder formulation of the active ingredient (concentration = 0.002 g/100 ml) and infected 48 h later with a conidia suspension of the fungus. The infected plants were incubated for 72 h at about 21 °C and high humidity and then kept in a greenhouse until typical leaf specks occurred. Fungicidal activity was evaluated 12 days after infection, based on the number and size of the specks.

In vivo tests using barley infected with *Erysiphe graminis*

Barley (*Hordeum aestivum* L.) plants were grown in a greenhouse as described for wheat.

Table I. *In vitro* inhibition (log I_{50}) or stimulation (log I_{500}) of metabolites of ergosterol biosynthesis in mycelia of *Ustilago maydis*, 24 h after treatment.

Metabolites[1]	Stereoisomers of etaconazole					Stereoisomers of propiconazole				
	12[2]	**l** *S/R*	**m** *S/S*	**n** *R/R*	**o** *R/S*	9[2]	**g** *R/S*	**i** *S/R*	**h** *S/S*	**k** *R/R*
	-log I_{500}[3] (stimulation)									
A	7.1	7.3	6.5	5.0	4.7	7.0	6.7	7.6	7.4	6.3
B	7.4	7.5	7.3	6.3	5.9	7.4	6.5	8.4	7.4	6.2
C	6.4	7.0	6.3	n.e.[5]	n.e.[5]	5.0	5.2	6.7	6.2	5.2
F	6.9	7.0	7.2	6.0	5.7	6.8	6.3	8.2	7.2	6.0
	-log I_{50}[4] (inhibition)									
D	6.8	6.9	6.9	5.5	5.2	6.6	6.5	7.2	6.8	6.1
E	5.7	5.7	5.7	4.6	4.3	4.8	4.6	5.6	5.7	4.4

[1] Metabolites assayed by gas chromatography. **A** 24-Methylendihydrolanosterol; **B** obtusifoliol; **C** 14α-methyl-ergosta-8,24(28)-dienol; **D** 22-dihydroergosterol; **E** ergosterol; **F** 14α-methyl-ergosta-8,24(28)-dien-3b,6a-diol.

[2] Commercial products cpds. No. 9 and 12, racemic mixture of all isomers.

[3] -log of concentration (mol/l) at which 500% increase of metabolite is observed (average of 3 determinations).

[4] -log of concentration (mol/l) at which 50% inhibition of metabolite is observed (average of 3 determinations).

In order to study the *residual protective activity* of the fungicide barley plants about 8 cm in height were sprayed with a spray mixture (0.002%) prepared from the active ingredient formulated as a wettable powder. The treated plants were dusted with conidia of the fungus 3–4 h later. The infected barley plants were then kept in a greenhouse at about 22 °C. The fungus attack was evaluated after 10 days.

For the experiments to investigate the *systemic activity* of the fungicide, barley plants about 8 cm in height were treated with a spray mixture (0.006 g/100 ml of soil) prepared from the active ingredient formulated as wettable powder. Care was taken that the spray mixture did not come in contact with parts of the plants above the soil. The treated plants were infected 48 h later with a conidia suspension of the fungus. The infected barley plants were then kept in a greenhouse at about 22 °C and evaluation of fungus infestation was made after 10 days.

Treatment of the *in vivo* fungicidal activity data

In vivo biological activities for inhibition of fungal growth as described above were originally in the form of bonitations (scale 1–9) at each of 5 different applied concentrations given in mg/l. Rather than trying to derive an IC_{50}, expressed in mol/l, we found it more appropriate to convert the raw data into a new 'activity index'-scale (AI) (Table II).

Although this scale is logarithmically spaced with respect to concentration (expressed in mg/l) it obviously does not exactly correspond to the log 1/C (molar) scale commonly used in linear free energy relationships. However, it proved to be advantageous to assess minor inconsistencies of the biological data and to take care of the fact that a number of compounds were either inactive at the highest dose or showed 100% effect at the lowest dose tested. Furthermore, it allows the sum or mean of activities to be calculated, in order to describe broad fungicidal activity. The use of concentrations in mg/l rather than in mol/l introduces only negligible errors as compared to the scatter of the biological data which is judged to be ± 1 AI-units for each species of fungi, or at least ± 2 to 3 units for the sum.

Table II. Definition of the "activity index", AI. The activity index AI corresponds to the lowest test concentration at which an effect of at least 80–95% (bonitation note < 3) is observed.

Activity index AI	Concentration [mg/l]
6	< 2 (very active)
5	2
4	6
3	20
2	60
1	200
0	> 200 (inactive)

Physicochemical parameters used in regression analysis

For some key compounds, marked by (*) in tables III to V *n*-octanol/water partition coefficients were determined by the flask-shaking method according to established procedures (OECD method No. 107, OECD [12]). The aqueous phase was M/15 phosphate buffer of pH 7.0. Based on the log P's of the measured compounds the other partition coefficients were calculated using lipophilic fragment constants from different sources [13–15]. Some fragments used were from our own data bank and had been derived from own measurements.

For the aromatic ortho-, meta- and para-substituents Hammett sigma constants were used to model their electronic effects. The size of the aromatic para-substituents were described by L and B_4, Verloop's parameters for the length and largest width of the van der Waals volume of the substituent [16]. As size parameter for the substituents R_3 to R_5 of the dioxolane and dioxane rings and to model dispersive interaction with a possible receptor site we chose MR, the molar refraction [17]. This parameter is also suitable to describe the volume effects of the disubstituted and bridged derivatives.

Particular substitution patterns of the aromatic rings, such as the presence of ortho- or meta-Cl atoms were described by indicator variables. Indicator variables were also used for special features of the dioxane and dioxolane rings, such as disubstitution, and for particular substituents. A total of 6 such discontinuous variables were tried in the final analysis. Multiple regression analyses were carried out with the program BMDP2R [18].

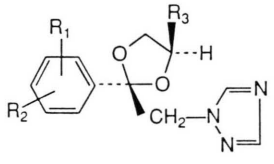
Results and Discussion

The inhibition of ergosterol biosynthesis in *Ustilago maydis* by the four isomers of etaconazole and propiconazole, as presented in Table I, shows a few

interesting facts: as two of the various methylated intermediates of the ergosterol pathway [5] are inhibited (**D**, 22-dihydroergosterol and **E**, ergosterol) the other four tested metabolites accumulate, as suggested in the literature [5, 19]. Furthermore it can be seen, that the 2*S*-isomers (*i.e.* isomers **a**, **b** and **h**, **i** resp.) are clearly more active than the 2*R*-isomers (*i.e.* **n**, **o** and **g**, **k** resp.) in respect to both the inhibition of metabolites **D** and **E** as well as for the accumulation of metabolites **A**, **B**, **C** and **F**. No significant difference in activity can be detected between the log I_{50}-values of the 2*S*,4*S*- and 2*S*,4*R*-isomers. Considering the commercial products, *i.e.* the racemic mixture of all isomers, it can be seen, that etaconazole is equally active as the two more active 2*S*-isomers. The *cis*-isomers of propiconazole on the other hand were more active than propiconazole itself. The results of the *in vitro* experiments with the stereoisomers allow some thoughts about the hydrophobic pocket of the cytochrome P-450 binding site into which the substituted dioxolane moiety protrudes. Computer modelling shows, that the four different isomers of propiconazole or etaconazole, when superposed with their triazole and benzene moieties, have their aliphatic R_3-substituents sticking into quite different directions. The hydrophobic dioxolane binding site must therefore provide ample space to accomodate such diverse configurations without dramatic change in activity. We have tried, by the use of a Hansch analysis, to elucidate whether the influence of the size of the aliphatic dioxolane substituents could be detected among other structural and physicochemical factors influencing the fungicidal activity.

The biological data of the *in vivo* fungicide tests, expressed in the form of the 'activity index' (AI), are shown in Tables III to V together with the lipophilicity parameter log *P*. The other variables used in the analysis have not been included in the printed tables. For increased clarity, the compounds were divided

Table III. Monosubstituted dioxolanes: structures, fungicidal activities and lipophilicities. Abbr.: Tr = 1,2,4-triazole, Ox = 1,2,4-oxadiazole. * = Experimental value.



≡ *"cis"*

No.	R_1	R_2	R_3	Activity index (6 = active, 0 = inactive) Pucc. res.	Cerc. res.	Erysiphe res.	Erysiphe sys.	Sum. act. indices exp.	Sum. act. indices calc. Eqn. (1)	log *P*
1	2-Cl	4-Cl	H	1	3	4	4	12	12.9	2.32*
2	H	H	H	0	1	3	2	6	5.3	0.83
3	4-Br	H	H	0	1	3	2	6	6.1	1.69
4	4-CH_3	H	H	0	1	3	2	6	6.6	1.55*
5	2-Cl	5-Cl	H	0	2	3	2	7	9.5	2.23
6	4-Cl	H	H	0	1	2	2	5	5.9	1.54
7	3-Cl	4-Cl	H	0	1	2	2	5	5.4	2.83
8	2-Cl	4-Cl	CH_3	2	4	5	4	15	12.9	2.51
9	2-Cl	4-Cl	$C_3H_7(n)$	6	5	5	4	20	15.2	3.55
10	2-Cl	4-Cl	$C_3H_7(n)$ *cis*	5	6	6	5	22	–	3.55
11	2-Cl	4-Cl	$C_3H_7(n)$ *trans*	5	4	6	3	18	–	3.55
12	2-Cl	4-Cl	C_2H_5	4	5	6	4	19	14.1	3.03*
13	2-Cl	4-Cl	C_2H_5 *cis*	4	6	5	4	19	–	3.03
14	2-Cl	4-Cl	C_2H_5 *trans*	2	5	5	3	15	–	3.03
15	2-Cl	4-Cl	$C_4H_9(n)$	4	4	5	2	15	16.4	4.07
16	2-Cl	5-Cl	CH_3	4	2	3	3	12	9	2.75
17	2-Cl	4-Cl	CH_3 *cis*	3	4	4	4	15	–	2.51
18	2-Cl	4-Cl	CH_3 *trans*	3	4	4	4	15	–	2.51
19	4-C_6H_5	H	CH_3	1	3	3	1	8	7.6	3.19
20	4-C_6H_5	H	C_2H_5	1	3	3	1	8	8.8	3.71*
21	4-C_6H_5	H	$C_3H_7(n)$	3	3	3	2	11	9.9	4.23

Table III. Continued.

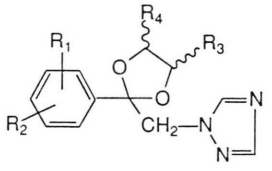
No.	R_1	R_2	R_3	Activity index (6 = active, 0 = inactive) Pucc. res.	Cerc. res.	Erysiphe res.	Erysiphe sys.	Sum. act. indices exp.	Sum. act. indices calc. Eqn. (1)	log *P*
22	2-Cl	4-Cl	CH_2OCH_3	4	5	5	6	20	21	2.21*
23	2-Cl	4-Cl	$CH_2OC_6H_5$	6	6	5	2	19	12.5	3.35
24	2-Cl	4-Cl	CH_2OH	0	1	4	3	8	4.3	1.76*
25	2-Cl	4-Cl	$CH_2OCH_2CH{=}CH_2$	4	3	4	3	14	12.1	2.89
26	2-Cl	4-Cl	$CH_2OCH_2CH_2OCH_3$	3	5	6	6	20	19	2.03*
27	2-Cl	4-Cl	$CH_2OC_2H_5$	4	4	6	4	18	12.7	2.73
28	2-Cl	4-Cl	$CH_2OC_4H_9(n)$	3	3	3	2	11	15	3.77
29	2-Cl	4-Cl	$CH_2OC_3H_7(n)$	3	5	6	3	17	13.6	3.25
30	2-Cl	4-Cl	CH_2Tr	2	3	3	3	11	11	2.65
31	2-Cl	4-Cl	$CH_2OC_6H_5C_6H_5$	1	1	3	0	5	–	5.01
32	2-Cl	4-Cl	$CH_2OC_6H_5C_6H_5$	1	1	1	0	3	–	5.01
33	2-Cl	4-Cl	$CH_2OC_6H_5OC_6H_5$	0	3	0	0	3	–	5.11
34	2-Cl	4-Cl	$CH_2CH_2CH_2CH_2OH$	0	0	0	1	1	4.7	2.42
35	H	H	CH_2Tr	1	0	0	0	1	3.6	1.23
36	2-Cl	4-Tr	C_2H_5	3	1	3	0	7	7.8	1.80
37	2-Cl	4-Tr	CH_3	0	0	1	2	3	8.4	1.28*
38	2-Cl	4-Cl	$C_3H_7(i)$	4	2	6	2	14	15.2	3.55
39	2-Cl	4-Cl	$C_4H_9(t)$	6	2	6	3	17	16.3	4.06
40	H	4-NO_2	C_2H_5	2	0	4	2	8	4.8	1.52*
41	H	4-NH_2	C_2H_5	0	0	0	0	0	2.3	0.57
42	H	4-CN	C_2H_5	0	1	3	3	7	3	1.23
43	H	4-C(=NH)NH_2	C_2H_5	0	0	0	2	2	2.4	0.85*
44	2-Cl	4-Br	C_2H_5	3	3	6	3	15	14.8	3.37
45	H	4-NCS	C_2H_5	0	1	0	0	1	–	2.95
46	H	4-$NHCONHCH_3$	C_2H_5	0	0	0	0	0	−0.9	0.62*
47	2-Cl	4-NO_2	C_2H_5	2	0	6	3	11	11.6	2.23
48	2-Cl	4-Br	$C_3H_7(n)$	6	3	6	3	18	16	3.89
49	H	4-$NHCSNHCH_3$	C_2H_5	0	0	1	1	2	−1.1	0.57
50	H	4-$NHCOCF_3$	C_2H_5	0	0	1	0	1	1.3	1.08*
51	H	4-Ox-3′-$C_6H_5Cl(p)$	C_2H_5	0	0	3	0	3	3.5	2.70
52	H	4-$NHCSNHC_3H_7(i)$	C_2H_5	0	0	0	0	0	2.1	1.59
53	H	4-$NHCSNHC_3H_7(n)$	C_2H_5	0	0	0	0	0	2.2	1.61
54	H	4-$NHCOC_6H_5NO_2(p)$	C_2H_5	0	0	0	0	0	1.3	2.17*
55	2-Cl	H	$C_3H_7(i)$	2	0	3	3	8	14.5	2.81
56	H	4-CH=C(CN)CN	C_2H_5	0	0	2	0	2	0.4	0.46
57	2-Cl	4-Cl	$CH_2OC_4H_9(n)$ *cis*	3	2	3	5	13	14.6	3.76
58	2-Cl	4-Cl	$CH_2OCH_2CH{=}CH_2$ *cis*	3	2	0	3	8	12.1	2.88
59	2-Cl	4-Br	C_2H_5	3	3	3	3	12	15.3	3.37
60	2-Cl	4-Cl	$CH_2SC_2H_5$	2	2	3	3	10	13.2	3.23

into three chemical groups: mono- and disubstituted dioxolanes, Tables III and IV, and 1,3-dioxanes, Table V.

The activity indices of the four different fungal species were found to be reasonably well correlated with each other with pairwise correlation coefficients between 0.62 and 0.89 in the case of the dioxolanes. Since in this study we were particularly interested to assess the fungicidal activity against a broad range of fungi, most calculations were made using the sum of the activity indices rather than the data for the individual species.

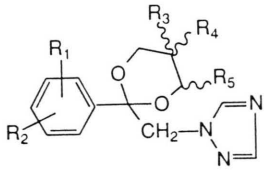
Fig. 4a (for the mono- and disubstituted dioxolanes) and Fig. 4b (for the dioxanes) show the dependence of the fungicidal activity on lipophilicity. It is apparent, particularly in the case of the dioxolanes, that activity increases with log *P* and seems to reach an optimum at a log *P* between 3 and 4. The dependence is less pronounced in the case of the 1,3-dioxanes. There are three dioxolanes, No. 31 to 33, with a high log *P* around 6 which show low activity. They all have either a diphenyl or diphenyl-ether substituent in position R_3. Since preliminary quantitative analyses could not clearly detect whether

Table IV. Disubstituted dioxolanes: structures, fungicidal activities and lipophilicities. * = Experimental value.



No.	R_1	R_2	R_3	R_4	Activity index (6 = active, 0 = inactive) Pucc. res.	Cerc. res.	Erysiphe res.	Erysiphe sys.	Sum. act. indices exp.	Sum. act. indices calc. Eqn. (1)	log *P*
61	2-Cl	4-Cl	CH_3	CH_3	5	4	4	4	17	15.9	2.89
62	4-C_6H_5	H	CH_3	CH_3	2	3	3	2	10	10.6	3.57
63	2-Cl	4-Cl	CH_3	C_2H_5	3	6	6	4	19	17.1	3.41*
64	2-Cl	4-Cl	$-(CH_2)_4-$		4	3	6	3	16	17.1	3.57
65	2-Cl	4-Cl	$-(CH_2)_4-$ (*trans*)		6	3	4	4	17	17.1	3.57
66	2-Cl	4-Cl	$-(CH_2)_4-$ (*cis*)		4	4	4	4	16	17.1	3.57

Table V. 1,3-Dioxanes: structures, fungicidal activities and lipophilicities. Abbr.: Im = imidazole. * = Experimental value.



No.	R_1	R_2	R_3	R_4	R_5	Activity index (6 = active, 0 = inactive) Pucc. res.	Cerc. res.	Erysiphe res.	Erysiphe sys.	Sum. act. indices	log *P*
67	2-Cl	4-Cl	H	H	H	3	4	4	3	14	2.85
68	2-Cl	4-Cl	CH_3	CH_3	H	3	5	5	3	16	3.89
69	2-Cl	4-Cl	CH_3	C_2H_5	H	3	6	5	2	16	4.41
70	2-Cl	4-Cl	CH_3	$C_3H_7(n)$	H	3	4	5	1	13	4.93
71	2-Cl	4-Cl	C_2H_5	C_2H_5	H	5	4	5	2	16	4.93
72	2-Cl	4-Cl	CH_3	CH_2OH	H	0	0	1	1	2	2.22*
73	2-Cl	4-Cl	CH_3	CH_2OH	H	0	0	1	1	2	2.10*
74	2-Cl	4-Cl	CH_3	$COOC_4H_9(n)$	H	0	1	2	1	4	4.20
75	2-Cl	4-Cl	CH_3	NO_2	H	3	1	1	1	6	2.27
76	2-Cl	4-Cl			H	0	1	1	0	2	1.87*
77	2-Cl	4-Cl	C_2H_5	CH_2OH	H	0	1	1	0	2	2.68
78	2-Cl	4-Cl		$.HNO_3$	H	3	2	3	2	10	3.47
79	2-Cl	4-Im	CH_3	CH_3	H	0	0	1	1	2	2.26*
80	2-Cl	4-Cl			H	3	0	0	0	3	3.83
81	4-C_6H_5	H	H	H	CH_3	0	3	4	0	7	3.71
82	4-c_6H_5	H	CH_3	CH_3	H	2	1	3	0	6	4.23

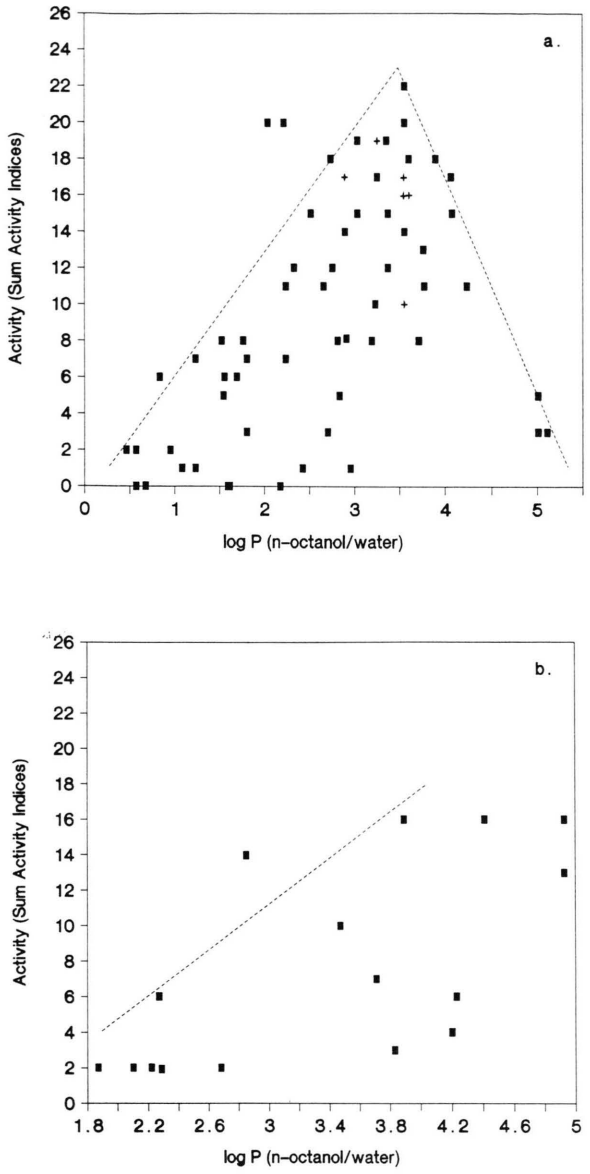


Fig. 4. Fungicidal activity, expressed as the sum of the activity indices (see text) *vs.* lipophilicity. a) Dioxolanes, squares: monosubstituted, crosses: disubstituted; b) dioxanes.

their low activity is due to their high lipophilicity or to their large size (these quantities are highly correlated for the aliphatic and aromatic R_3-substituents of this series), they were omitted from the final regression equation (1). If they would have been left in, their contribution could have been acknowledged either by a separate indicator variable or by a square term in log P or MR_{R3}. We have also omitted from the regression analyses the *cis*- and *trans*-isomers of the CH_3-, C_2H_5, and $C_3H_7(n)$-derivatives (Nos. 10, 11, 13, 14, 17 and 18) because their activities (sum) do not diverge significantly from the activities of the mixtures of the *cis*- and *trans*-isomers. The *cis*-isomers do, however, always show a higher activity than the *trans*-isomers. We have also omitted the 4-CNS-derivative (Nr. 45) from the final regression equation because preliminary analyses had shown that this compound is an outlier. For reasons not further investigated (probably decomposition) its activity is much smaller than would be expected.

Stepwise development of multiple regression of the remaining 56 mono- and disubstituted dioxolanes leads to the following 'best' Hansch-equation:

$$\begin{aligned} AI = 5.02 &+ 3.28\ (\pm 0.53) \cdot \log P - 1.14\ (\pm 0.35) \cdot I \\ &- 0.12\ (\pm 0.07) \cdot MR_{R3} + 4.40\ (\pm 1.23) \cdot I \\ &- 4.72\ (\pm 1.93) \cdot I_{m\text{-}Cl} + 9.85\ (\pm 2.18) \cdot I_{R\text{-}O} \\ &- 5.97\ (\pm 2.17)\, I_{R\text{-}OH} + 2.45\ (\pm 1.32) \cdot I_{di\text{-}R} \end{aligned}$$

$$n = 56, \quad r = 0.909, \quad s = 2.89, \quad F_{1,47} = 27.8.$$

The most important single variable is log P, with a positive regression coefficient, indicating higher activity with increasing log P. Of the other continuous variables tested, only L_{X4}, the variable for the length of the *para*-substituent of the aromatic moiety and MR_{R3}, the size parameter for the dioxolane substituent entered into the regression, both with negative coefficients, indicating a decrease of activity when either the aromatic parasubstituents or the dioxolane-substituents become too large. The size parameters are also responsible for the fact, that no square term in log P appears in the regression equation as might be expected from Fig. 4a. Apparently, the activity-decreasing effect of high lipophilicity is loaded onto L_{X4} and MR_{R3}.

Interestingly, σ, the Hammett constants of the aromatic substituents, appear to have no significant influence on activity, since these variables do not explicitly enter into the regression equation. The main reason for this is, that most of the electronic effect of the aromatic substituents is modeled by the two indicator variables, one for the presence of an ortho-Cl substituent, $I_{o\text{-}Cl}$, with a strongly positive contribution to the activity (4.4 AI-units) and one for a meta-Cl substituent, $I_{m\text{-}Cl}$, with an equally large negative coefficient. A further reason for the apparently small electronic influence of the aromatic sub-

stituents is the following: while the para-substituents vary rather widely from electron donating substituents like NH_2 to strong electron acceptors like NO_2, all non-halogen substituents which are mostly in compounds with lower activity than the halogens, are larger than Cl and are thus modelled by the size parameter L_{X4} discussed above.

The influence of the electronic effect of the aromatic moiety and of the lipophilicity of the whole molecule on activity can be illustrated by Fig. 5a: all

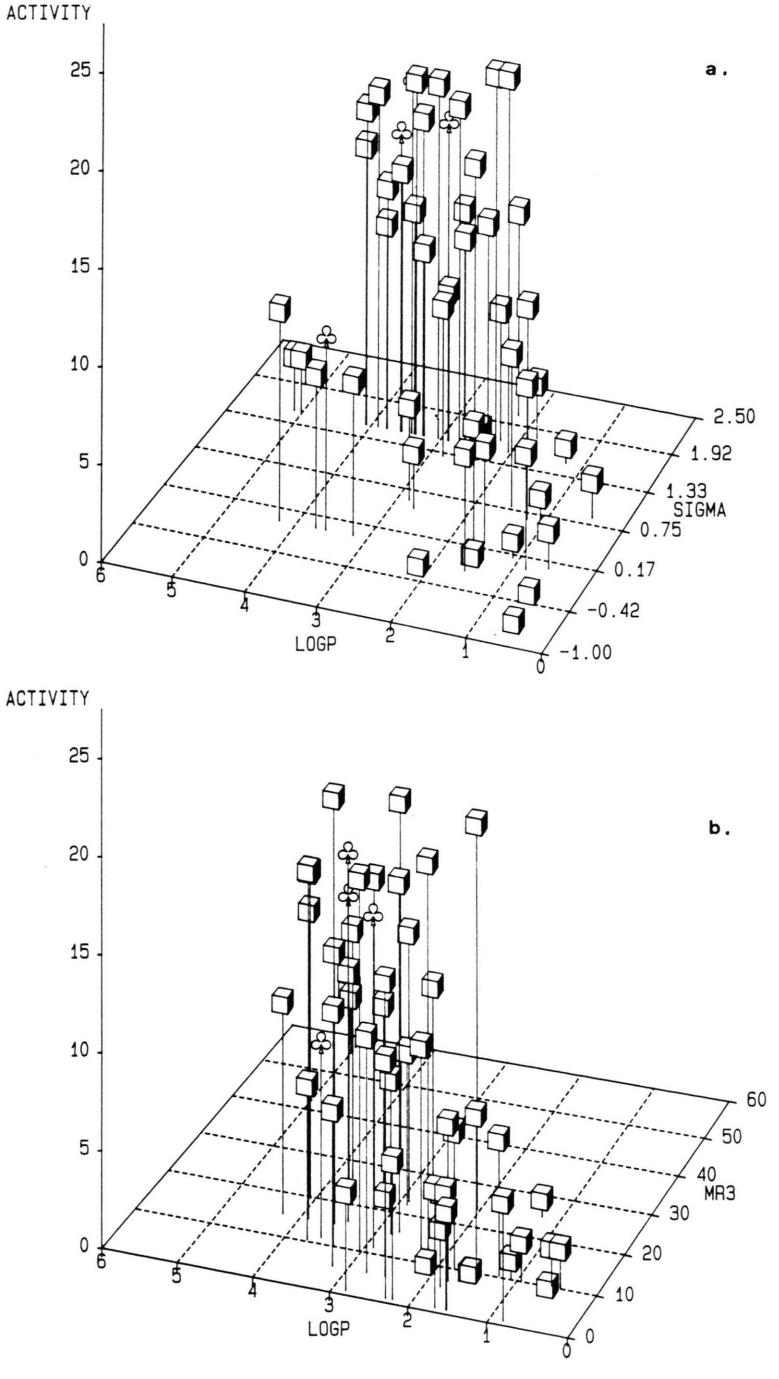


Fig. 5. Fungicidal activity (sum of the activity indices) for mono- (cubes) and disubstituted (clubs) dioxolanes. a) Against lipophilicity and Hammett sigma constant; b) against lipophilicity and molar refraction of the dioxolane substituents.

highly active compounds lie near $\sigma = 1.5$ which corresponds to the sum of σ of 2,4-dihalogen. The effect of the size of the dioxolane substituents is shown in Fig. 5b. The highest activities are reached when MR_{R3} is between 10 and 20, simultaneously with lipophilicities near the optimum of log $P = 3.5$. Obviously, etaconazole ($MR_{R3} = 10.3$) and propiconazole (MR_{R3}) are near this optimum.

Two interesting compounds with unusually high activities can be identified in the upper left of Fig. 4a: compounds Nr. 22 and 26, both with a methoxy end group on R_3, are clearly outside the envelope drawn around the majority of the data points. The coefficient of the indicator variable in equation (1), introduced for these two derivatives, shows that their activities are higher by almost 10 AI-points than would be expected from the regression of the majority of compounds. We attempted to further improve their activity by increasing their lipophility. However, adding additional methylene groups in the side chain or methyl or ethyl groups in 5-position of the dioxolane ring proved unsuccessful, possibly due to steric effects.

This study has shown that the racemic commercial product TILT® is the fungicidally most active derivative of this series of dioxolane and dioxane triazoles. It has also shown that in both etaconazole and propiconazole, the 2*S*-isomers were the most active compounds and there was practically no difference in the log I_{50}-values of the 2*S*,4*S*- and the 2*S*,4*R*-isomers. In the case of etaconazole the more active isomers were as active as the racemic mixture.

It appears difficult to optimize the chemical structure further, staying within the framework of structural features used so far. The high *in vivo* activity, together with a remarkably low phytotoxicity have already made this product a very successful fungicide in different important crops such as small grain cereals, peanuts, grape vines, apples etc.

Acknowledgements

The authors thank J. Gaudin for technical assistance.

[1] F. J. Schwinn, Pestic. Sci. **15,** 40 (1984).
[2] H. Buchenauer, Biochem. Physiol. **7,** 309 (1977).
[3] M. J. Henry and H. D. Sisler, Pestic. Sci. **12,** 98 (1981).
[4] P. Gadher, E. I. Mercer, B. C. Baldwin, and T. E. Wiggins, Pestic.-Biochem. Physiol. **19,** 1 (1983).
[5] E. Ebert, J. Gaudin, W. Muecke, K. Ramsteiner, C. Vogel, and H. Fuhrer, Z. Naturforsch. **38c,** 28 (1983).
[6] T. Kato, Chemistry of Plant Protection (1), Springer Verlag, Berlin, Heidelberg 1986.
[7] G. Van Reet, J. Heeres, L. Wals, Janssen AG., DOS **2,** 551–560 (1974).
[8] J. Heeres, Pestic. Sci. **15,** 268 (1984).
[9] C. Vogel, T. Staub, G. Rist, and E. Sturm, Proc. 5th IUPAC Pesticide Chemistry Congress, Pergamon Press, Kyoto 1983.
[10] M. Winitz, L. Bloch-Frankenthal, Nobuo Jzumiya, Sanford M. Birnbaum, Carl G. Baker, and Jesse P. Greenstein, J. Am. Chem. Soc. **78,** 2423 (1956).
[11] H. Buchenauer, Z. Pflanzenkrankheiten, Pflanzenschutz **84,** 286 (1977).
[12] OECD Guidelines for Testing of Chemicals, Section 1, Physical-Chemical Properties. Bureau of Information, Paris 1981.
[13] C. Hansch and A. J. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology. Wiley, New York 1979.
[14] R. F. Rekker and H. M. deKort, Eur. J. Med. Chem. **14,** 479 (1979).
[15] Pomona College Medicinal Chemistry Project, Computer Program CLOGP, Release 3.2, Claremont, Calif. 91711, 1983.
[16] A. Verloop, W. Hoogenstraten, J. Tipker, in: E. J. Ariëns (ed.), Drug Design, **Vol. 7,** p. 165, Academic Press, New York 1976.
[17] A. K. Ghose and G. M. Crippen, J. Chem. Inf. Comput. Sci. **27,** 21 (1987).
[18] BMDP Statistical Software, U. of California Press, Berkeley, 1983.
[19] N. N. Ragsdale, in: M. R. Siegel and H. D. Sisler (eds.), Antifungal compounds, **Vol. II,** p. 333, Marcel Dekker Inc., New York and Basel 1977.
[20] M. Taton, P. Ullmann, P. Benveniste, and A. Rahier, Pesticide Biochemistry and Physiology **30,** 178–189 (1988).