MOLECULAR CALCULATIONS AND THERMODYNAMICAL CONSIDERATIONS ON THE SOLUBILITY ENHANCEMENT OF TRIFLUMIZOLE BY CYCLODEXTRIN COMPLEXATION

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ABSTRACT

Complexation of pesticides (fungicides, herbicides, insecticides, etc.) with cyclodextrins results in advantageous modifications of their properties like e.g. enhanced solubility and therefore a better activity combined with a reduced application concentration. The activity of the fungicide Triflumizole encapsulated in β-cyclodextrin was investigated and thermodynamical parameters of the association process were determined.

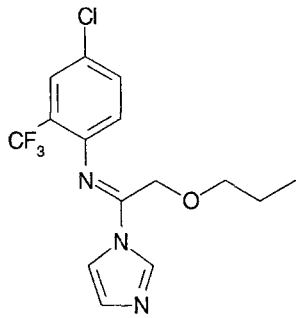
1. INTRODUCTION

The solubilizing effect of various cyclodextrins is of particular interest in the pharmaceutical industry and chemical technology. Solubility enhancement of pesticides and improved activity is caused by complexation of mostly hydrophobic compounds with convenient host molecules. Such inclusion complexes are studied widely [1-4]. The stability of the inclusion complexes is determined by the molecular shape of the guest molecule and the complementary molecular surface of the cavity additional to attractive van der Waals and electrostatic forces. As a quantitative measure of the complex stability the association constant K can be taken together

Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **25**: 237–241, 1996.

with the corresponding thermodynamical parameters. The solvent is of main importance for the building and the stability of the inclusion complexes.

Triflumizole (TF), (Picture 1), is a fungicidal substance with systemical effect against plant mycosis like powder mildew, rust and scab (black spot-desease) on cereals, arboricultures and vinicultures.



Picture 1: Triflumizole

Due to the poor wettability and extremely low solubility in water Triflumizole is to be applicated on plants as suspension. To improve the solubility as well as the activity of the conazole fungicide solid Triflumizole/β-cyclodextrin complexes were prepared by precipitation in a molar ratio of 1:2, leading to complete encapsulation of the active ingredient (AI).

2. MATERIALS AND METHODS

Triflumizole, (E)-4-chloro-α,α,α-trifluoro-N-(1-imidazol-1-yl-2-propoxy-ethylidene)-*o*-toluidine (JUPAC), (E)-1-[1-[[4-chloro-2-(tri-fluoro-methyl)phenyl]-imino]-2-propoxyethyl]-1H-imidazole (C.A.), CAS Nr. *99 387-89-0,* is a systemic fungizide and has a curative and protective action against Gymnosporangium and Venturia spp. in pome fruits, against powdery Erysiphaceae in fruits and vegetables, against Fusarium, Fulvia and Monilinia spp. as well as Helmintosporium, Tilletia and Ustilago spp. in cereals [3]. The compound was obtained from Nippon Soda Co. Ltd. (Japan) with a purity of >99%. It forms colourless crystals, m.p. 63,5º, v.p. 0.0014 mPa (25ºC); solubilities (20ºC): in water: 12,5mg/l; in trichloromethane: 2.22g/l; hexane 17.6g/l. β-cyclodextrin was provided by Roquette Fréres (Lestrem, France) as Kleptose® with a humidity of 14%(w/w).

The stability constant of the association complex between Triflumizole and β-cyclodextrin was estimated by different methods: By an iterative procedure, where calculated and experimentally determined extinctions at various concentrations are fitted together [5], and by another method based on the monitoring of changes in solubility of Triflumizole by addition of various concentrations of β-cyclodextrin. These measurements were carried out by suspending excess amounts of Triflumizole in solutions of β-cyclodextrin in the concentration range of 4.10^{-4} to 6.10^{-3}M. After stirring the samples at constant room temperature for some hours, the concentration of dissolved Triflumizole was determined again by electron absorption spectroscopy.

3. RESULTS AND DISCUSSION

The saturation concentration of the compound in water was estimated as $4{,}2.10^{-5}$ mol/l. The electronic absorption spectra of Triflumizole and the association complex are different, aqueous solutions of Triflumizole show absorptions of 234,5nm (ε=25800) and 293,8 (ε=4050), which shift to 362,2nm (ε=25400) and 3990nm (as shoulder) for the inclusion complex. The respective association constant determined by different methods was estimated to 470±20 M^{-1} in aqueous solution. The overall reaction enthalpy and the overall reaction entropy were calculated from the temperature dependence of the equilibrium constant (ΔH = -22,4 kJ/mol; ΔS = -24 J/K.mol). Small amounts of organic cosolvents like ethanol or dioxane diminish the association constant drastically parallel to an enhanced solubility of pure Triflumizole. The driving force of the inclusion of Triflumizole into the hydrophobic interior of β-cyclodextrin is therefore the hydrophobicity of the substrate. Complexation decreases the total hydrophobic surface compared to the isolated molecules and leads to a rather stable associate.

The structures of the host-guest complex were estimated by molecular mechanics calculations, using the MM3 force field [5-7]. A broad variety of conformations can be observed for the cyclodextrin rim, due to the single bonds between the glucose subunits, which allow some deformation motions of the cone. The flexibility of the cyclodextrin ring structures as well as of the association complexes were analyzed by a stochastical search [4]. The association process itself was investigated by modified simulated annealing procedures [4, 8].

The fungicide activity of the Triflumizole/β-cyclodextrin complex was investigated on apples on small plot trials with 4 replications. The treatment was done with a 20 l handsprayer 7 times

in the growing period using 0,5 to 0,9 l water per tree. After the growing period the apples were harvested and representative samples were taken to determine the attack of scab. The biological activity of β-cyclodextrin containing preparations against leaf scab on appletrees was also tested in comparison to conventional commercial products. The results of complex activity tests against fruit scab on apples and against leaf scab on appletrees are shown in tables 1 and 2.

	1. Replication %attached (total)	2. Replication %attached (total)	3. Replication %attached (total)	4. Replication %attached (total)	Average %attached (total)
Triflumizole/β-CD-complex (10mg% AI)	1,36 (661)	1,18 (680)	1,48 (677)	1,27 (551)	1,32 (643)
Condor ® (12,5mg% AI)	3,77 (531)	1,71 (702)	2,99 (703)	0 (449)	2,12 (596)
untreated	15,5 (625)	-	-	-	15,5 (625)

Table 1: Activity against fruit scab on apples *(Arlet, Gold Delicius, Jonagold, Empire)*

	1. Replication % leaf surface attached	2. Replication % leaf surface attached	3. Replication % leaf surface attached	Average % leaf surface attached
Triflumizole/β-CD-complex (10mg% AI)	1,15	0,17	0,27	0,53
Tank mix (35mg% AI)	0,57	0,26	0,83	0,55
untreated	17,93	18,10	17,75	17,93

Table 2: Activity against leaf scab on appletrees *(Gold Delicius)* Tank mix = 0,0125% Triflumizole + 0,0225% Dodine (standard mix)

The in vivo investigations demonstrate that clathrates have the same activity against leaf scab at a level of 0,01% AI as a standard tank mix of Triflumizole and Dodine with a combined AI rate of 0,035%.

By applicating Triflumizole/β-cyclodextrin complexes against fruit scab on different sorts of apples again complexes showed a higher fungicide activity compared with a standard formulation.

The data from these studies suggest that a treatment with fungicides as cyclodextrin complexes can save drug substance; as fungicide/cyclodextrin association complexes show higher biological activity than the pure drug, ecological as well as economical benefits can be achieved by using low pesticide doses in plant-protecting formulations.

4. CONCLUSION

The fungicide Triflumizole forms inclusion complexes with β-cyclodextrin caused by the hydrophobicity of the compound and the interior of the host molecule. The equilibrium constant K of the association reaction is in such a order of magnitude, that the solubility of Triflumizole in water is considerably enhanced and that the availbility of the free compound is given. These properties make the association complex useful for a commercial application as fungicide, which has been proven by extensive activity tests.

ACKNOWLEDGEMENTS

The authors want to thank Fa. Kwizda for financial support.

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