RISK ASSESSMENT OF FUNGAL BIOLOGICAL CONTROL AGENTS: *BEAUVERIA BRONGNIARTII* AND ITS METABOLITE OOSPOREIN - NEEDS AND DEEDS

Christoph Seger,[1,2] Daniela Erlebach,[2] Ulrich Griesser,[3] Waltraud Wimmer,[1] Sonja Sturm,[1] Hermann Stuppner,[1] and Hermann Strasser[2]

[1]*Institute of Pharmacy, Department of Pharmacognosy, Leopold-Franzens University of Innsbruck, Innrain 52, A-6020 Innsbruck*
[2]*Institute of Microbiology, Leopold-Franzens University of Innsbruck, Technikerstraße 25, A-6020 Innsbruck*
[3]*Institute of Pharmacy, Department of Pharmaceutical Technology, Leopold-Franzens University of Innsbruck, Innrain 52, A-6020 Innsbruck*

email: Christoph.Seger@uibk.ac.at

INTRODUCTION

Beauveria brongniartii (Ascomycota, anamorph of Clavicipitales) is known to be a very selective and highly virulent entomopathogenic fungus used in the control of *Melolontha melolontha* (chockchafer; Coleoptera, Scarabaeidae).

This fungal biological pest control agent (BCA) is an environmentally friendly alternative to pesticides. It's usage will help reducing or phasing out the use of toxic pesticides (i.e. methyl bromide, chlorpyrifos).

NEEDS

Currently, proper risk assessment is one of the major hurdles in the registration process of BCAs in the EU (covered by the EU Directive 91/414/EEC).

Risk-assessment of fungal BCAs needs detailed knowledge on the temporal and spatial distribution of both the BCA and it's major secreted secondary metabolites in the environment (Strasser, Vey & Butt, 2000).

A prerequisite for the estimation of the mobility and behaviour of a chemical substance in a biological matrix is the detailed knowledge of the physico-chemical parameters of the questioned analyte.

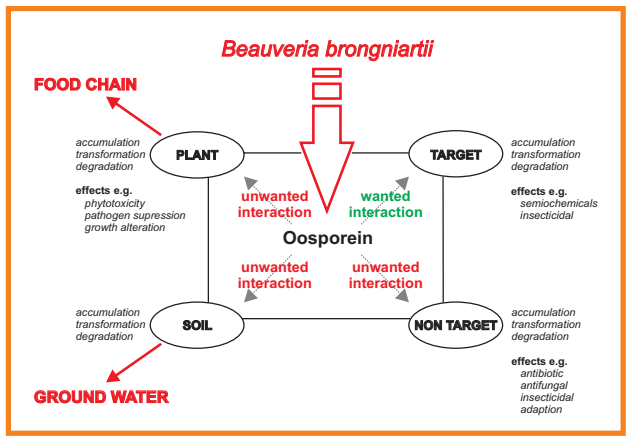
The major metabolite of *B. brongniartii*, the hydroxybenzoquinone derivative oosporein is poorly characterized and its fate in biological matrices is largely unknown (Strasser et al., 2000).

DEEDS

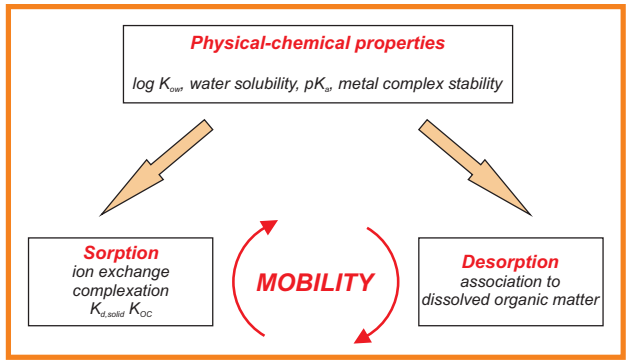
With a set of simple experiments the pH and temperature dependence of the solubility and lipophily of an analyte can be tested. By monitoring these experiments with HPLC, the stability of the analyte can be evaluated.

RESULTS

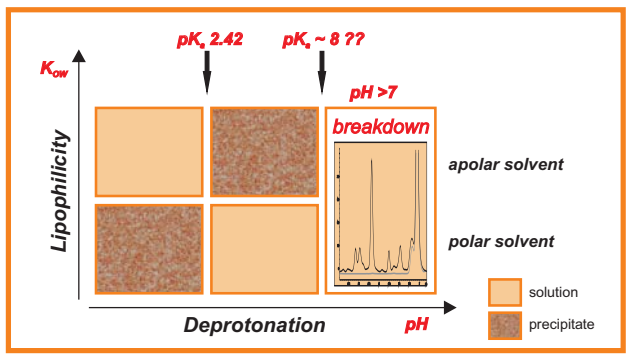
- Oosporein is a weak organic acid (pK_a = 2.42).
- It is lipophilic at acidic pH.
- It is hydrophilic at neutral and alkaline pH.
- It's water solubility increases with pH and temperature.
- It is highly unstable at alkaline pH.
- It readily adsorbes to glass surfaces.



Distribution pathways of a BCA metabolite demonstrated on oosporein.



Connection of physico-chemical parameters and mobility.



pH dependence of oosporein solubility and stability.

ACKNOWLEDGEMENT

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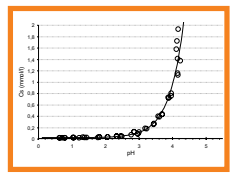
Strasser, H., Vey, A. & Butt, T. (2000)
Biocontrol Science and Technology, **10**, 717-735.

Strasser, H., Abendstein, D., Stuppner, H. & Butt T. (2000)
Mycological Research, **104**, 1227-1233.



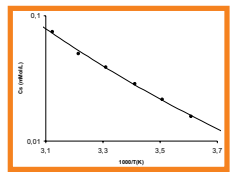
Solubility (Cs) of oosporein in citrat/HCl buffer as function of the pH at 20 °C.
Circles represent measured values, overlayed with the calculated Henderson- Hasselbalch curve.

C_w = 24±0.03 Mol
pKa = 2.42±0.02

CONSEQUENCES

The water solubility of oosporein is a function of the pH.

The solubility at pH = 1 (stomach) is very low (25 Mol = 7.8 ppm).



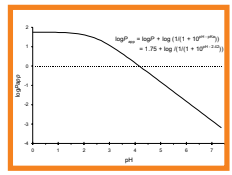
Solubility (Cs) of oosporein in citrat/HCl buffer (pH 1,23) as function of the temperature.
Dots represent measured values, overlayed with a calculated nonlinear Van't Hoff curve.

H_s = 26.0 kJ/mol at 25 °C
lnC_s = -123.3+2678.9*T⁻¹+19.5*lnT

CONSEQUENCES

The water solubility of oosporein is a function of the temperature.

The solubility at pH = 1.23 and 20 °C is 28 Mol and increases with a rate of about 1 Mol/°C.



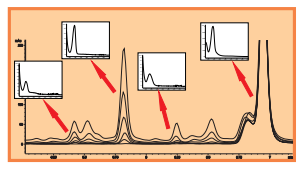
Octanol/water partition coefficient of oosporein: pH dependence of the logP.

$logP_{ow}$ = 1,73±0.03 at pH 1.2
P_{app} = 53,7±4.1 at pH 1.2

CONSEQUENCES

The lipophilicity of oosporein is a function of the pH; it decreases with rising pH. Oosporein is not lipid soluble at a pH > 7.

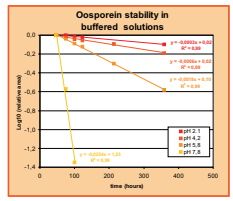
The passive diffusion of oosporein through biological membranes can be excluded at neutral and alkaline pH. It is possible in acidic media.



Oosporein breakdown monitored with a chromatographic method.

Incubation in Britton-Robinson buffer (pH = 5.8 at 43 °C)

HPLC: gradient elution
solvent: ACN/H_3O(0.1% TFA)



Oosporein breakdown curves at differnt pH values.

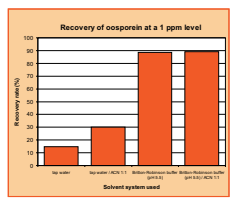
Britton-Robinson buffers at 43 °C.

$_{pH=2.1}$ = 42 days
$_{pH=4.2}$ = 21 days
$_{pH=5.8}$ = 7 days
$_{pH=7.8}$ = 0.5 days

CONSEQUENCES

The stability of oosporein is a function of the pH; it decreases with rising pH.

Oosporein is highly instable at a pH > 7 - it can not sustain in alkaline environments.



Recovery of oosporein from different solvent systems.

Very low recovery rate of oosporein with tap water.

CONSEQUENCES

Oosporein readily binds to glass surfaces by complex formation with abundant cations.

This complex formation can be broken by the Britton-Robinson buffer.

The addition of organic solvents does not influence the results.