Bioactive secondary metabolites from fruiting bodies of Higher Fungi

National Conference on Mushrooms and Mushroom Cultivation in Ethiopia, May 13-16,2008

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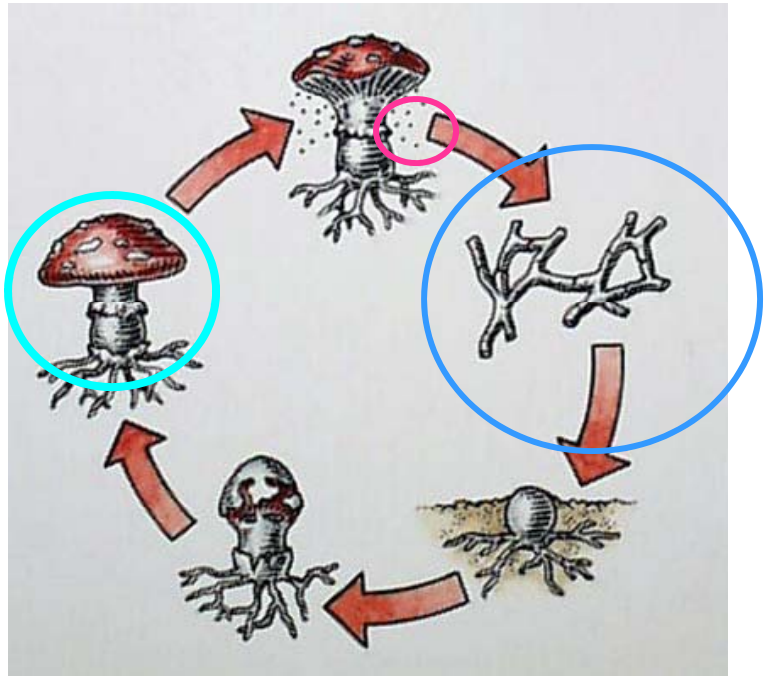
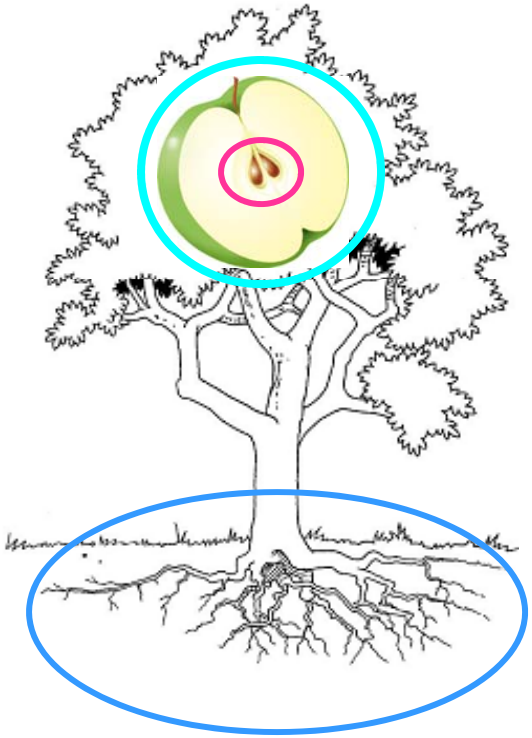
- **Introduction**
- **Secondary metabolites from fruiting bodies of *Hygrophorus***
- **Secondary metabolites from fruiting bodies of *Cortinarius***
- **Bioactivity of fungal secondary metabolites**

Fungal fruiting bodies as a source of natural products

Why dealing with fungi ?

- Evolutionary very old organism
- First multicellular organisms which are successfully growing on soil
- Life cycle: different environment, different enemies





fungi: most of the life cycle (mycelium) under the grassroot

reproductive organs (fruiting bodies): ~ 1 month over the grassroot

e.g.: *Armillaria bulbosa*: 1 500 years old, over 10 000 kg, 15 ha (Nature 356: 428-431)

Armillaria ostoyae: 2 400 years old, 900 ha

Fungal fruiting bodies as a source of natural products

Why dealing with fungi ?

- Evolutionary very old organism
- First multicellular organisms which are successfully growing on soil
- Life cycle: different environment, different enemies
- High specialisation (ectomycorrhiza, endomycorrhiza, endophytes)

→ **wide spectrum of new compounds expected**

Bioassay guided isolation of secondary metabolites





Extraction → Sample processing → *In vitro* bioactivity screening → active, nontoxic → Isolation and structure elucidation

Isolation and structure elucidation → Pure compounds

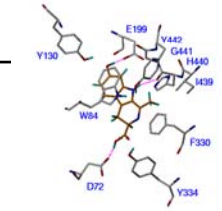
Pure compounds → Synthesis, derivatization

Pure compounds → *In vitro / in vivo* testing

Pure compounds → pharmaco-kinetic studies

Pure compounds → Biosynthesis





Synthesis, derivatization → Pure compounds

In vitro / in vivo testing → Pure compounds

pharmaco-kinetic studies → Pure compounds

Pure compounds → active → **lead**


Hygrophorones A – G from

Hygrophorus spp. Sect. Olivaceoumbrini

H. persoonii

H. olivaceoalbus

H. latitabundus

H. pustulatus

Genus *Hygrophorus*

- engl.: wax caps
- Tricholomataceae (Basidiomycetes)

- ~ 60 species
- symbionts (mycorrhiza) with trees

- fruiting bodies strong slimy surface

- various colour reactions of the stipes

- **field observation:**
  rarely attacked by parasites (pathogenic fungi, yeasts,...)

→ **assumption: bioactive secondary metabolites as preform defense**

Hygrophorus in Europe

Sect. **Hygrophorus** – Subsect. Chrysodonti

H. chrysodon

Gelbzahn-Schneckling

Sect. **Hygrophorus** – Subsect. Pallidini

H. penarius

Trockener Schneckling

Sect. **Hygrophorus** – Subsect. Hygrophorus

H. eburneus

Elfenbein-Schneckling

H. cossus

Verfärbender Schneckling

H. hedrychii

Birkenschneckling

H. gliocyclus

Schleimberingter Schneckling

H. carpini

Hainbuchen-Schneckling

H. chrysaspis

Sect. **Pudorini** – Subsect. Erubescentes

H. erubescens

Rasiger Purpurschneckling

H. russula

Purpur-schneckling

11 *H. capreolarius*

Weinroter Schneckling

Sect. **Pudorini** – Subsect. Pudorini

H. poetarum

Isabellrötlicher Schneckling

H. nemoreus

Wald-Schneckling

H. pudorinus

Orange-Schneckling

Sect. **Discoidei**

H. discoideus

Braunscheibiger Schneckling

H. unicolor

Orangefalber Schneckling

H. lucorum

Lärchen-Schneckling

H. hypothejus

Frostschneckling

Sect. **Olivaceoumbrini** – Subsect. – Olivaceoumbrini

H. olivaceoalbus

Natterstieliger Schneckling

H. persoonii

Olivgestiefelter Schneckling

H. latitabundus

Großer Kiefern-Schneckling

Sect. **Olivaceoumbrini** – Subsect. – Tephroleuci

H. pustulatus

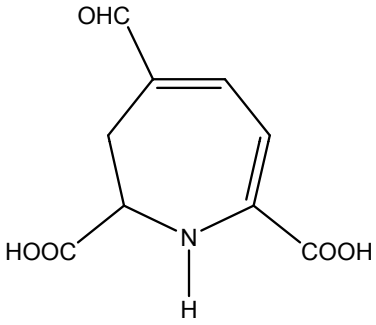
Schwarzpunktierter Schneckling

H. agathosmus

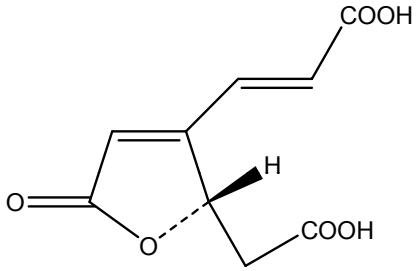
Mehliger Schneckling

Previous works on metabolites from *Hygrophorus*

- Fugmann et al. (1985): muscaflavin (1) and hygrophoric acid (2) in *H. aureus*, *H. hypothejus*, *H. lucorum* and *H. speciosus*
- Wood et al. (2003): 3-Chloroindole (3) in *H. paupertinus*
- Qu et al. (2004): Hygrophamide (4) in *H. eburneus*



1



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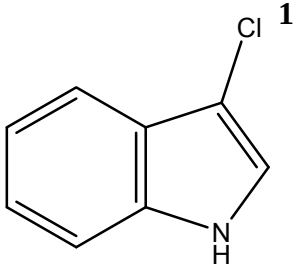

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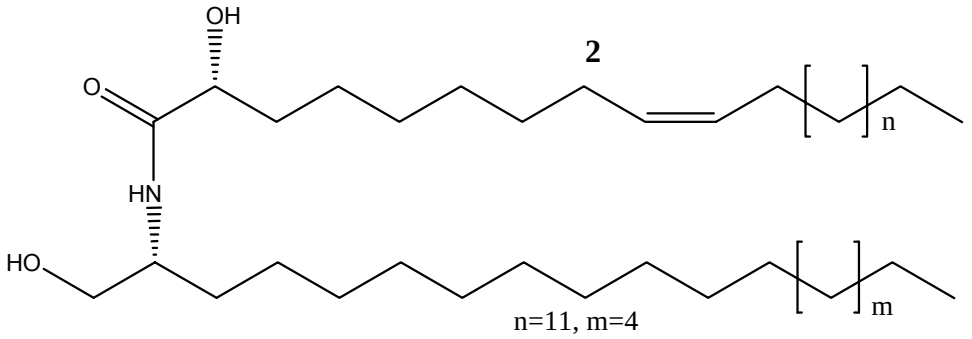


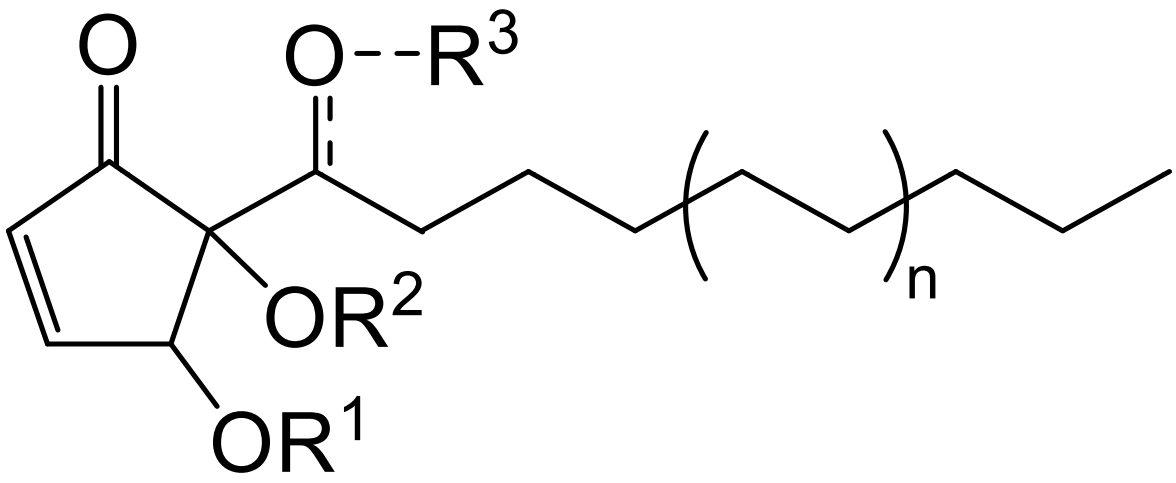
4

Fugmann, B. (1985) – Neue niedermolekulare Naturstoffe aus Höheren Pilzen. Dissertation, Universität Bonn

Wood, W., Smith, J., Wayman, K., Largent, D. (2003) – Indole and 3-chloroindole: the source of the disagreeable odor of *Hygrophorus paupertinus*. Mycologia 95(5), 807 – 808

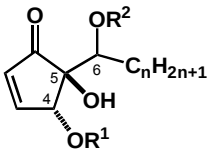
Qu, Y., Zhang, H., Liu, J. (2004) – Isolation and Structure of a new ceramide from the Basidiomycete *Hygrophorus eburnesus*. Z. Naturforsch. 59b, 241 - 244

Hygrophorones A – G from *Hygrophorus* spp.

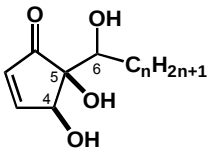


Lübken, T., Schmidt, J., Porzel, A., Arnold, N., & Wessjohann, L. (2004) - Hygrophorones A–G: fungicidal cyclopentenones from *Hygrophorus* species (Basidiomycetes). Phytochemistry, 65, 1061-1071.
Lübken, T., Arnold, N. & Wessjohann, L. Hygrophorone und deren Derivate. – PCT/EP/05001957

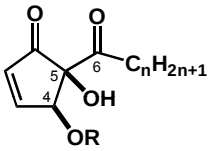
Hygrophorones A – G from *Hygrophorus* spp.



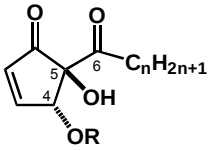
10	4,6-Di-*O*-acetyl-Hygrophoron A[12]	R^1 = Ac	R^2 = Ac	n = 12	*H. persoonii*
11	4-*O*-Acetyl-Hygrophoron A[12]	R^1 = Ac	R^2 = H	n = 12	*H. persoonii*
12	6-*O*-Acetyl-Hygrophoron A[12]	R^1 = H	R^2 = Ac	n = 12	*H. persoonii*
13	4,5-Di-*O*-acetyl-Hygrophoron A[14]	R^1 = Ac	R^2 = Ac	n = 14	*H. persoonii*
14	4-*O*-Acetyl-Hygrophoron A[14]	R^1 = Ac	R^2 = H	n = 14	*H. persoonii*
15	6-*O*-Acetyl-Hygrophoron A[14]	R^1 = H	R^2 = Ac	n = 14	*H. persoonii*



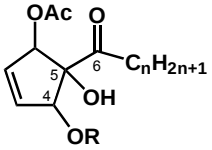
16	Hygrophoron B[14]	n = 14	*H. olivaceoalbus*
17	Hygrophoron B[16]	n = 16	*H. olivaceoalbus*



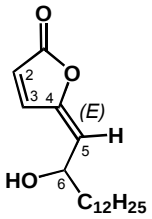
18	4-*O*-Acetyl-Hygrophoron C[12]	R = Ac	n = 12	*H. pustulatus*
19	Hygrophoron C[12]	R = H	n = 12	*H. pustulatus*



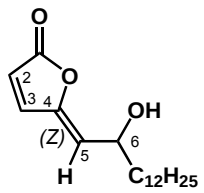
20	4-*O*-Acetyl-Hygrophoron D[12]	R = Ac	n = 12	*H. latitabundus*
21	Hygrophoron D[12]	R = H	n = 12	*H. latitabundus*
22	4-*O*-Acetyl-Hygrophoron D[14]	R = Ac	n = 14	*H. latitabundus*



23	1,4-Di-*O*-acetyl-Hygrophoron E[12]	R = Ac	n = 12	*H. latitabundus*
24	1,4-Di-*O*-acetyl-Hygrophoron E[10]	R = Ac	n = 10	*H. latitabundus*
25	1,4-Di-*O*-acetyl-Hygrophoron E[14]	R = Ac	n = 14	*H. latitabundus*
26	1-*O*-Acetyl-Hygrophoron E[12]	R = H	n = 12	*H. latitabundus*
27	1-*O*-Acetyl-Hygrophoron E[10]	R = H	n = 10	*H. latitabundus*



28 Hygrophoron F[12]
H. persoonii



29 Hygrophoron G[12]
H. persoonii

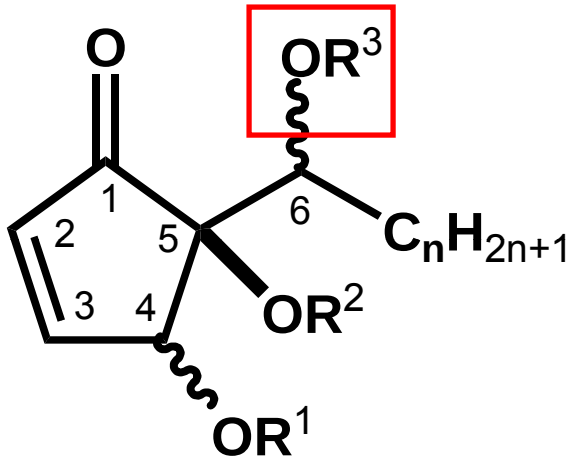
H. persoonii

H. olivaceoalbus

H. pustulatus

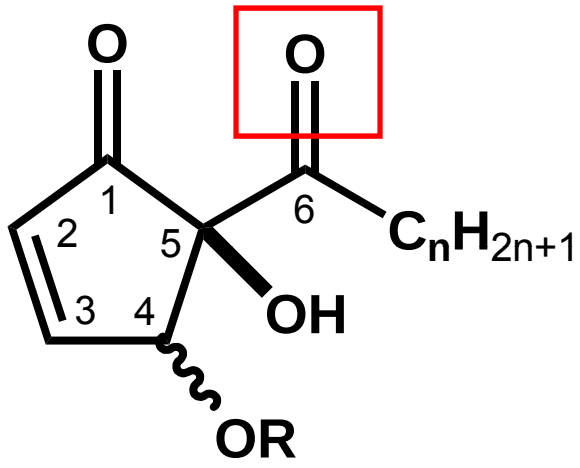
H. latitabundus

Structures of Hygrophorones



R^1, R^2, R^3 = H, Ac

Type I



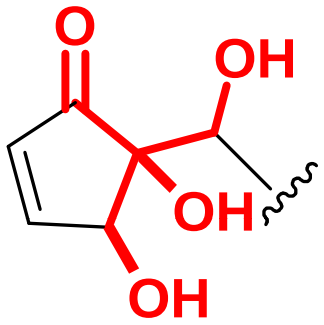
R = H, Ac

Type II

Lübken, T., Arnold, N., Wessjohann, L., Böttcher, C. & Schmidt, J. (2006) - Analysis of fungal cyclopentenone derivatives from *Hygrophorus* spp. by liquid chromatography/electrospray-tandem mass spectrometry. J. Mass Spectrometry 41 (3), 361 – 371.

Relation between structural features and negative ion ESI-CID mass spectra

Hygrophorones Type I

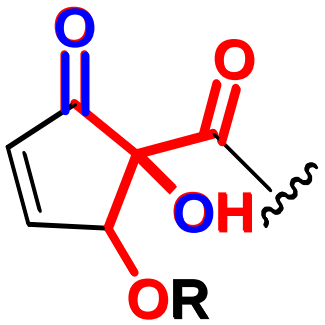


$[M-H-C_3H_4O]^-$

$[M-H-C_4H_4O_2]^-$

$[M-H-C_5H_6O_2]^-$

Hygrophorones Type II

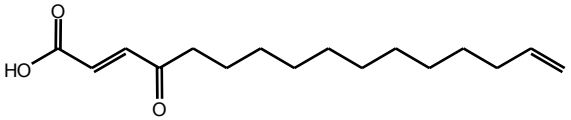


$[M-H-CO]^-$

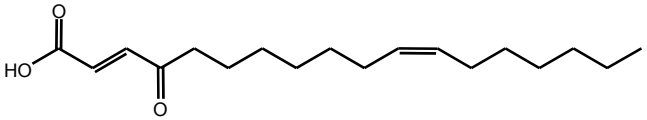
$[M-H-CO_2]^-$

Lübken, T., Arnold, N., Wessjohann, L., Böttcher, C. & Schmidt, J. (2006) - Analysis of fungal cyclopentenone derivatives from *Hygrophorus* spp. by liquid chromatography/electrospray-tandem mass spectrometry. J. Mass Spectrometry 41 (3), 361 – 371.

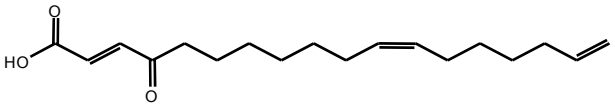
4-Oxo-2-alkenoic Fatty Acids



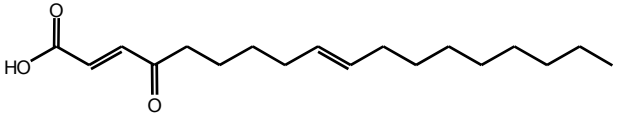
(*E*)-4-oxhexadeca-2,15-dienoic acid



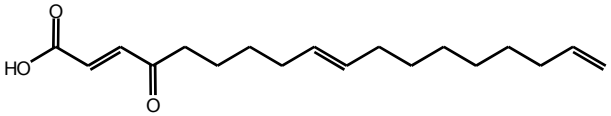
(2*E*,11*Z*)-4-oxooctadeca-2,11-dienoic acid



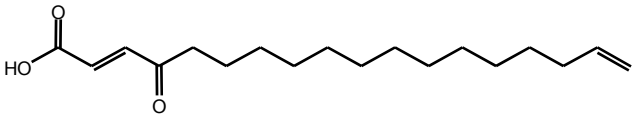
(2*E*,11*Z*)-4-oxooctadeca-2,11,17-trienoic acid



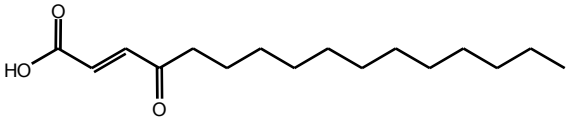
(2*E*,9*E*)-4-oxooctadeca-2,9-dienoic acid



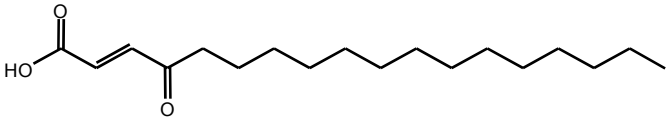
(2*E*,9*E*)-4-oxooctadeca-2,9,17-trienoic acid



(*E*)-4-oxooctadeca-2,17-dienoic acid



(*E*)-4-oxohexadeca-2-enoic acid



(*E*)-4-oxooctadeca-2-enoic acid

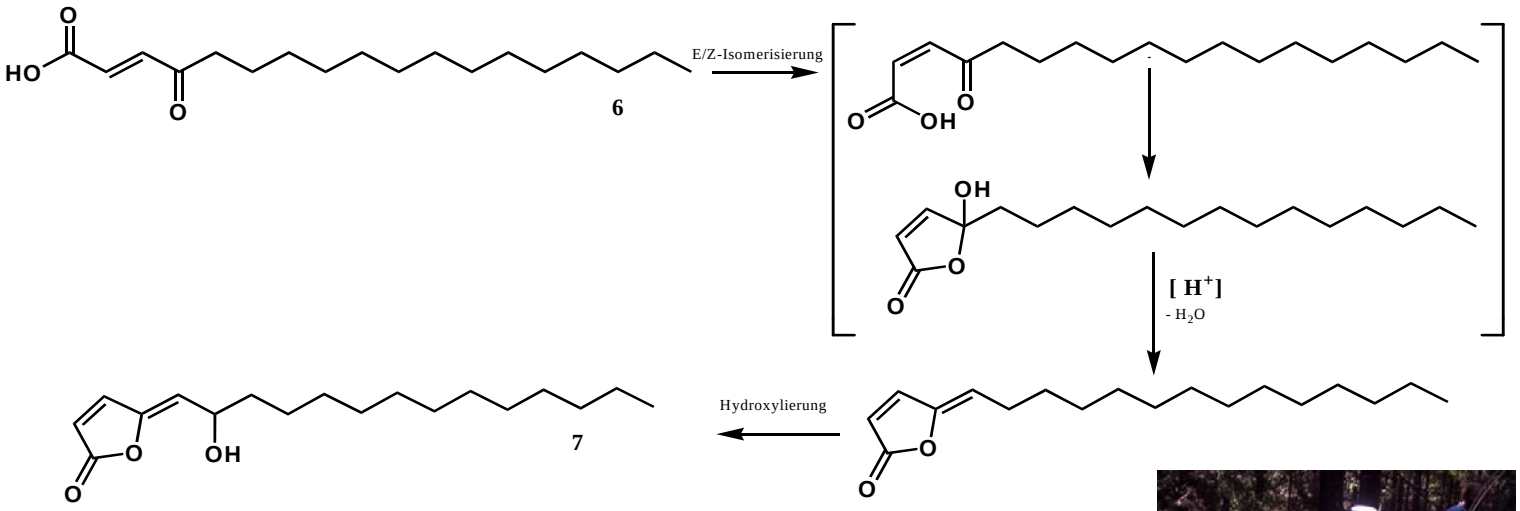
H. eburneus

Teichert, A., Lübken, T., Schmidt, J., Porzel, A., Arnold, N.& Wessjohann, L. (2005) - Unusual bioactive 4-oxo-2-alkenoic fatty acids from *Hygrophorus eburneus*. Z. Naturforsch. 60b, 25 - 32

Teichert, A., Lübken, T., Kummer, M., Besl, H., Haslberger, H. & Arnold, N. (2005) - Bioaktive Sekundärmetaboliten aus der Gattung *Hygrophorus* (Basidiomycetes). Z. Mykol., 71/1, 53 - 62

Proposed biosynthesis

(**6**) potential biosynthetic precursor of Hygrophoron G[12] (7) ?

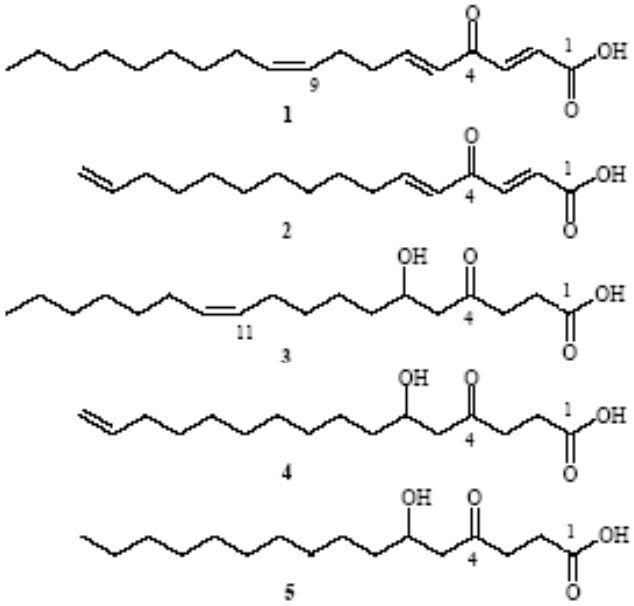


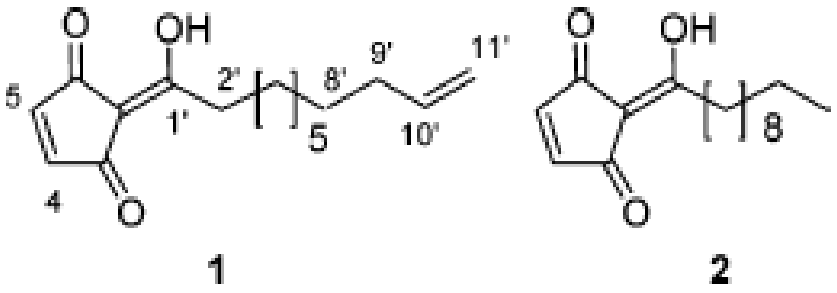
Feeding experiments with synthetic ^{13}C labelled fatty acids
→ **no incorporation detectable** (low concentration, weather conditions,...

Group Vidari (2006, 2007)

4-Oxo Fatty Acids from *Hygrophorus discoxanthus*

Acylcyclopentenediones from of *Hygrophorus chrysodon*

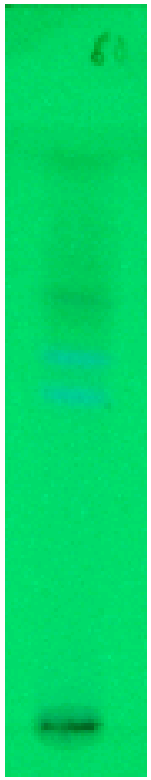




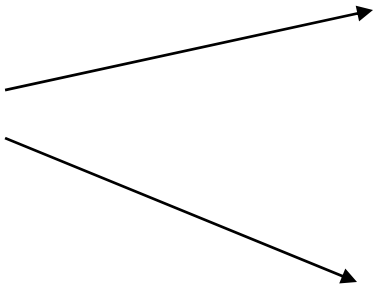
Gianluca Gilardoni, Marco Clericuzio, Solveig Tosi, Giuseppe Zanoni, and Giovanni Vidari (2007) - Antifungal Acylcyclopentenediones from Fruiting Bodies of *Hygrophorus chrysodon J. Nat. Prod.*, *70,* 137-139

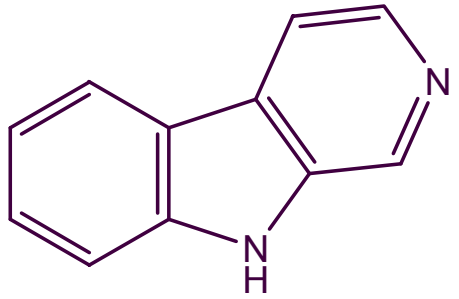
Gilardoni, G.; Clericuzio, M.; Marchetti, A.; Vita Finzi, P.; Zanoni, G.; Vidari, G. *Nat. Prod. Commun.* 2006, New oxidized 4-Oxo Fatty Acids from Hygrophorus discoxanthus. *Nat. Prod. Commun, 1,* 1079-1084.

Occurence of *β*-carboline alkaloids harmane and norharmane in *Hygrophorus* spp.



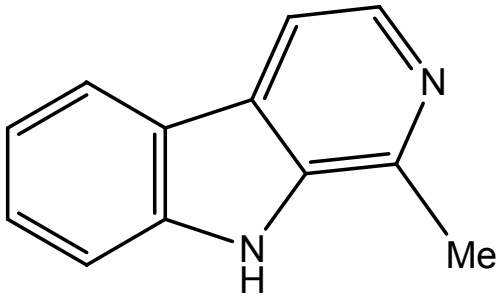
uv 254 nm ***uv 365 nm***





9*H*-pyrrido[3,4-*b*]indole

norharmane



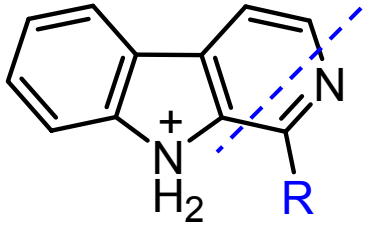
1-Methyl-9-*H*-carboline

harmane

Axel Teichert, Â., Schmidt, J., Kuhnt, C., Huth, M., Porzel, A., Wessjohann, L., Arnold, N. (2008) - Determination of β -carboline alkaloids in fruiting bodies of Hygrophorus spp. by liquid chromatography/electrospray ionisation tandem mass spectrometry. Phytochem. Analysis., in press.

Main Fragmentation of Harmane and Norharmane

1: Harmane (R=Me)
2: Norharmane (R=H)



- RCN

$[M+H-RCN]^+$

1, **2**: *m/z* 142

$[M+H]^+$ **1**: *m/z* 183
2: *m/z* 169

evidenced by
HR-ESI-CIDMS
(QqTOF)

- Me

$[M+H-Me]^+$

1: *m/z* 168

- HCN

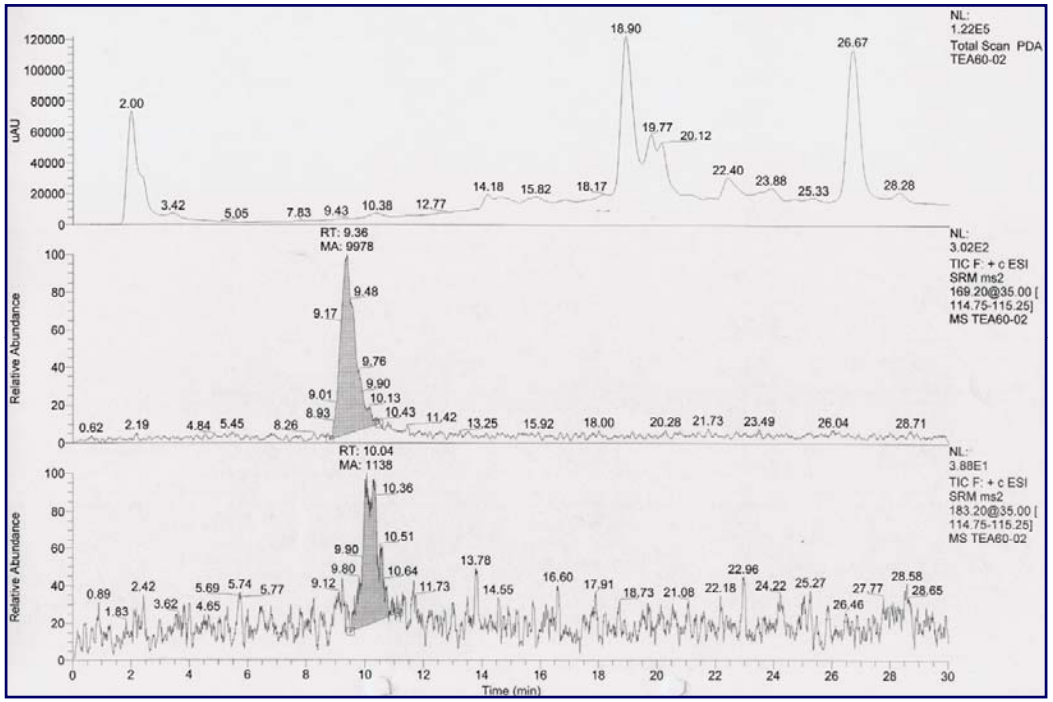
$[M+H-RCN-HCN]^+$

1, **2**: ***m/z* 115 (100%)**

Axel Teichert, Â., Schmidt, J., Kuhnt, C., Huth, M., Porzel, A., Wessjohann, L., Arnold, N. (2008) - Determination of β -carboline alkaloids in fruiting bodies of Hygrophorus spp. by liquid chromatography/electrospray ionisation tandem mass spectrometry. Phytochem. Analysis., in press.

Evidence for the co-occurrence of harmane and norharmane in 28 investigated *Hygrophorus* species by LC/ESI-SRM

Species	Norharman pg µL^{-1}	Harman pg µL^{-1}
H. agathosmus	1,04	0,30
H. carpinii	5,81	2,71
H. chrysaspis	3,06	4,85
H. chrysodon	15,84	0,94
H. cossus	16,98	1,25
H. dichrous	0,07	0,07
H. discoideus	31,04	2,31
H. discoxanthus	0,82	1,62
H. eburneus	95,62	8,62
H. erubescens	1,85	1,42
H. fuscoalbus	0,60	0,23
H. glyocyclus	0,91	0,86
H. hedrychii	3,82	0,65
H. hyacinthinus	3,44	5,47
H. hypothejus	13,16	1,68
H. latitabundus	8,96	1,83
H. lucorum	2,61	1,92
H. marzuolus	2,56	2,43
H. nemoreus	0,89	1,22
H. olivaceosalbus	3,81	0,42
H. penarius	8,50	5,63
H. persoonii	46,46	11,10
H. poetarum	4,13	0,83
H. pudorinus	0,54	0,85
H. pustulatus	2,39	3,91
H. russula	0,48	0,64
H. speciosus	0,78	0,53
H. unicolor	0,98	0,79



→ **harmane and norharmane - a chemotaxonomic marker**

Axel Teichert, Â., Schmidt, J., Kuhnt, C., Huth, M., Porzel, A., Wessjohann, L., Arnold, N. (2008) - Determination of β -carboline alkaloids in fruiting bodies of Hygrophorus spp. by liquid chromatography/electrospray ionisation tandem mass spectrometry. Phytochem. Analysis., in press.

Hygrophorus hyacinthinus Quel.

(very rare species)

daylight

uv light (365 nm)

Isolation

MeOH crude extract

↓

Diaion

↓

Sephadex LH20

↓

prep. HPLC, RP18

MeOH
365 nm

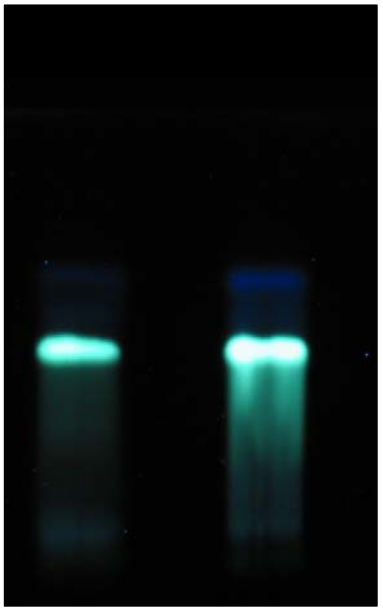
TLC 365 nm

HR-MS: *m/z* 245.092 (calc. for $C_{13}H_{13}O_3N_2$, $[M+H]^+$, 245.092)

m/z 201.102 (calc. for $C_{12}H_{13}O_1N_2$, $[M+H]^+$, 201.102) → $[M+H-CO_2]^+$

Similarities to *Cortinarius brunneus* – identical substances!

H. hyacinthinus

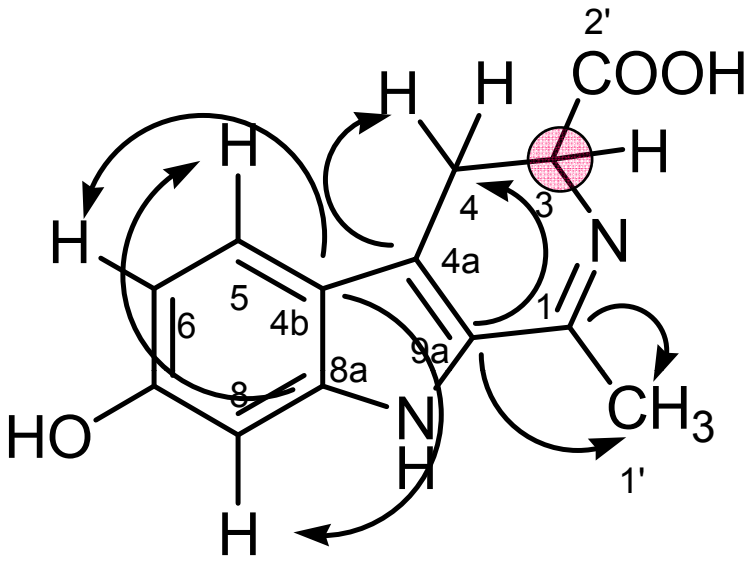


TLC: BuOH:HOAc:H_2O, 4:1:1

Cortinarius brunneus

Teichert, A., Schmidt, J., Porzel, A., Arnold, N., & Wessjohann, L. - Brunneins A – D, β-carboline alkaloids from Cortinarius brunneus (Basidiomycetes). *J. Nat. Prod.*, 70(9), 1529-1531.

^{13}C NMR



Brunnein A

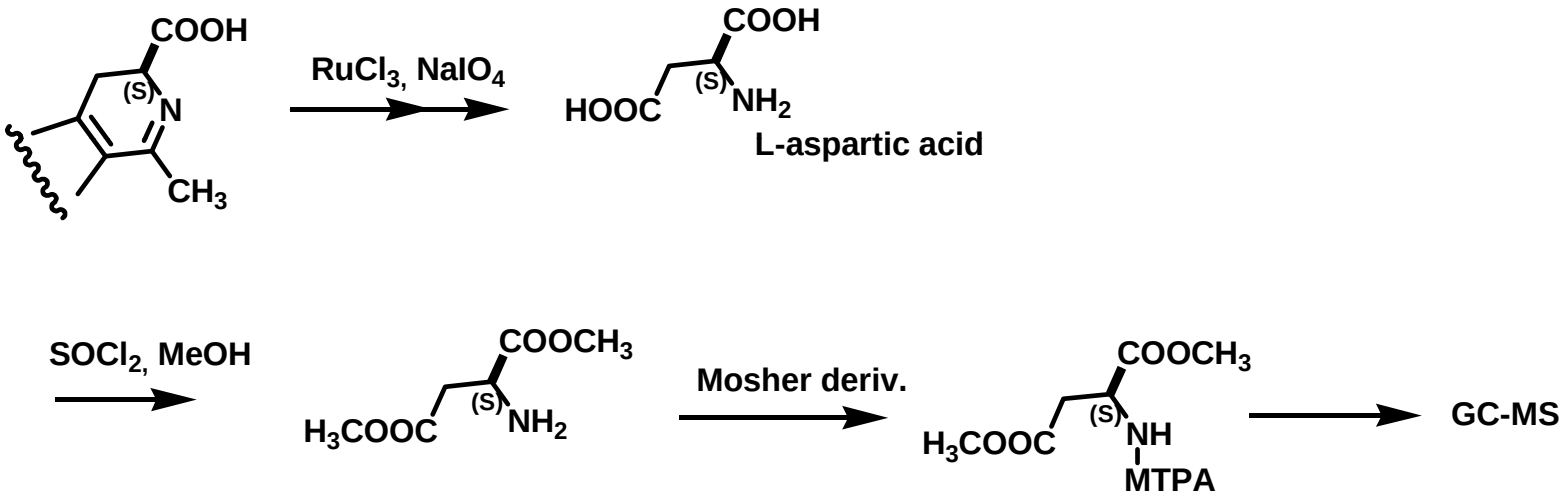
13 carbons: *i.a.* 6 quaternary C

1 chiral center → H-3$_{ax}$

Brunnein A: configuration at C-3

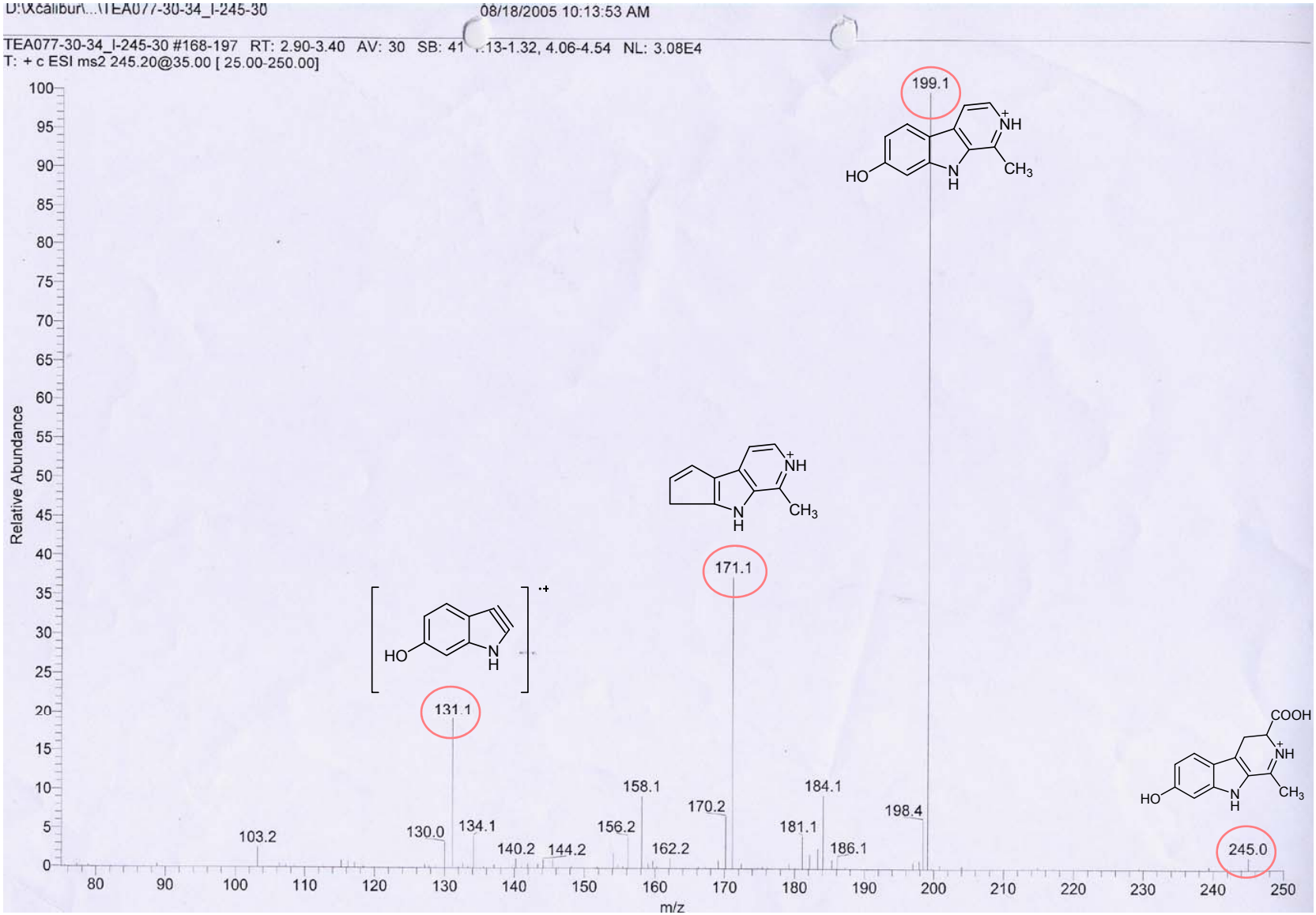
- CD measurement
- oxidative degradation and GC-MS analysis

→ **S-configuration, proposed biosynthetic precursor: L-tryptophane**



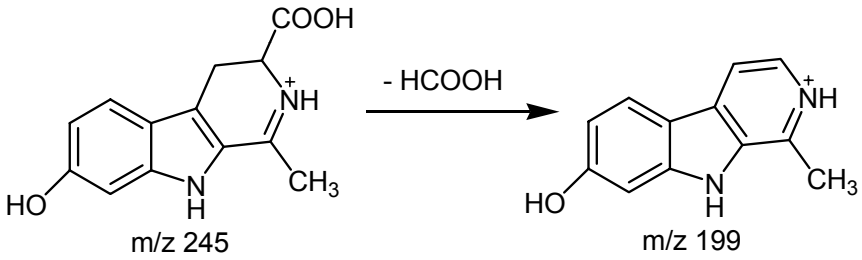
Bringmann, G., God, R & Schäfer, M. (1996) – An improved degradation procedure for determination of the absolute configuration in chiral isochinoline and ß-carboline derivatives. Phytochemistry 43, 1393-1403.

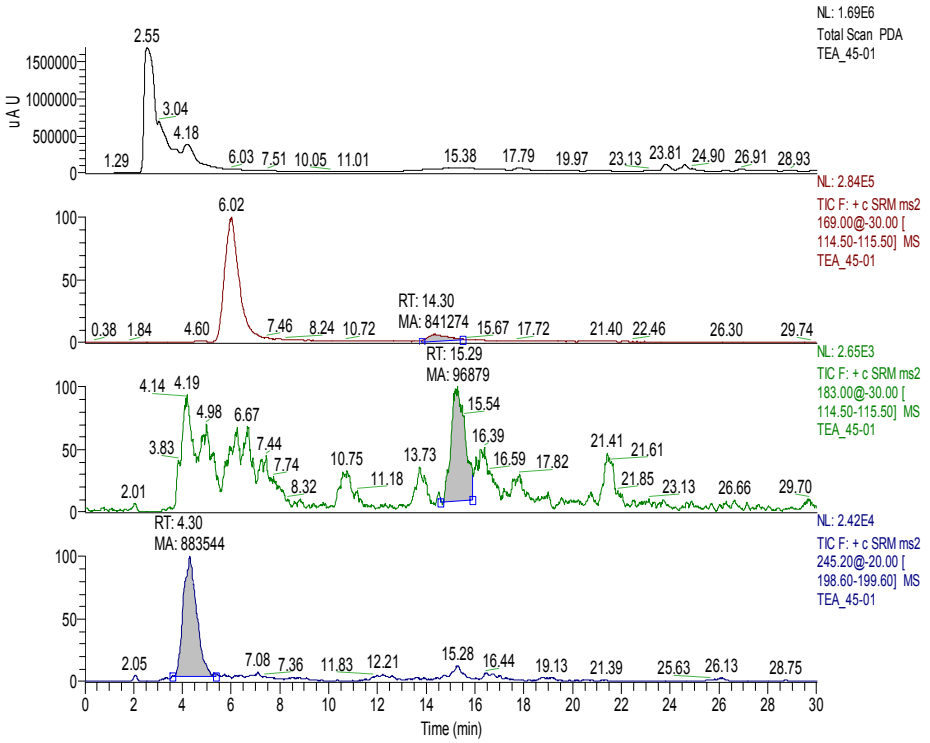
Brunnein A: CID mass spectrum from *LC-ESI-MS/MS*



Occurence of Brunnein A in *Hygrophorus* spp.

- SRM measurements:
- Using main CID MS fragmention of Brunnein A:





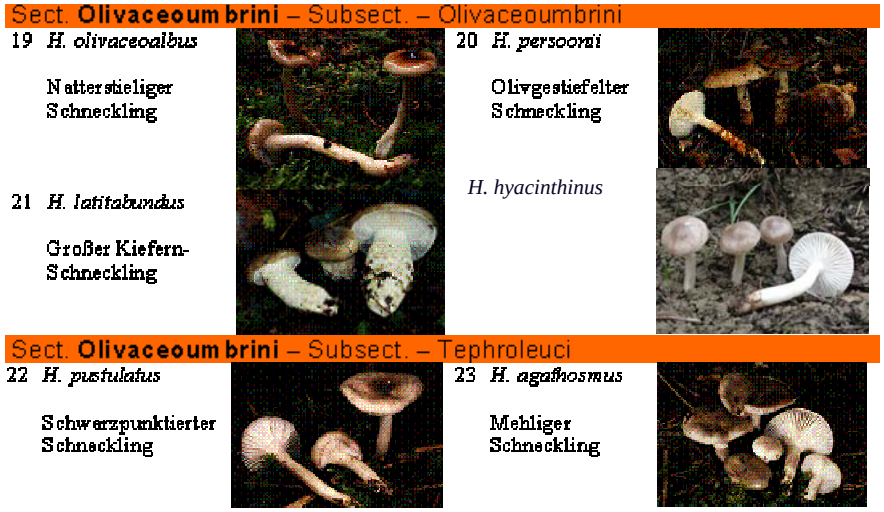
Axel Teichert, Â., Schmidt, J., Kuhnt, C., Huth, M., Porzel, A., Wessjohann, L., Arnold, N. (2008) - Determination of β -carboline alkaloids in fruiting bodies of Hygrophorus spp. by liquid chromatography/electrospray ionisation tandem mass spectrometry. Phytochem. Analysis., in press.

Occurence of Brunnein A in *Hygrophorus* spp.

Results:

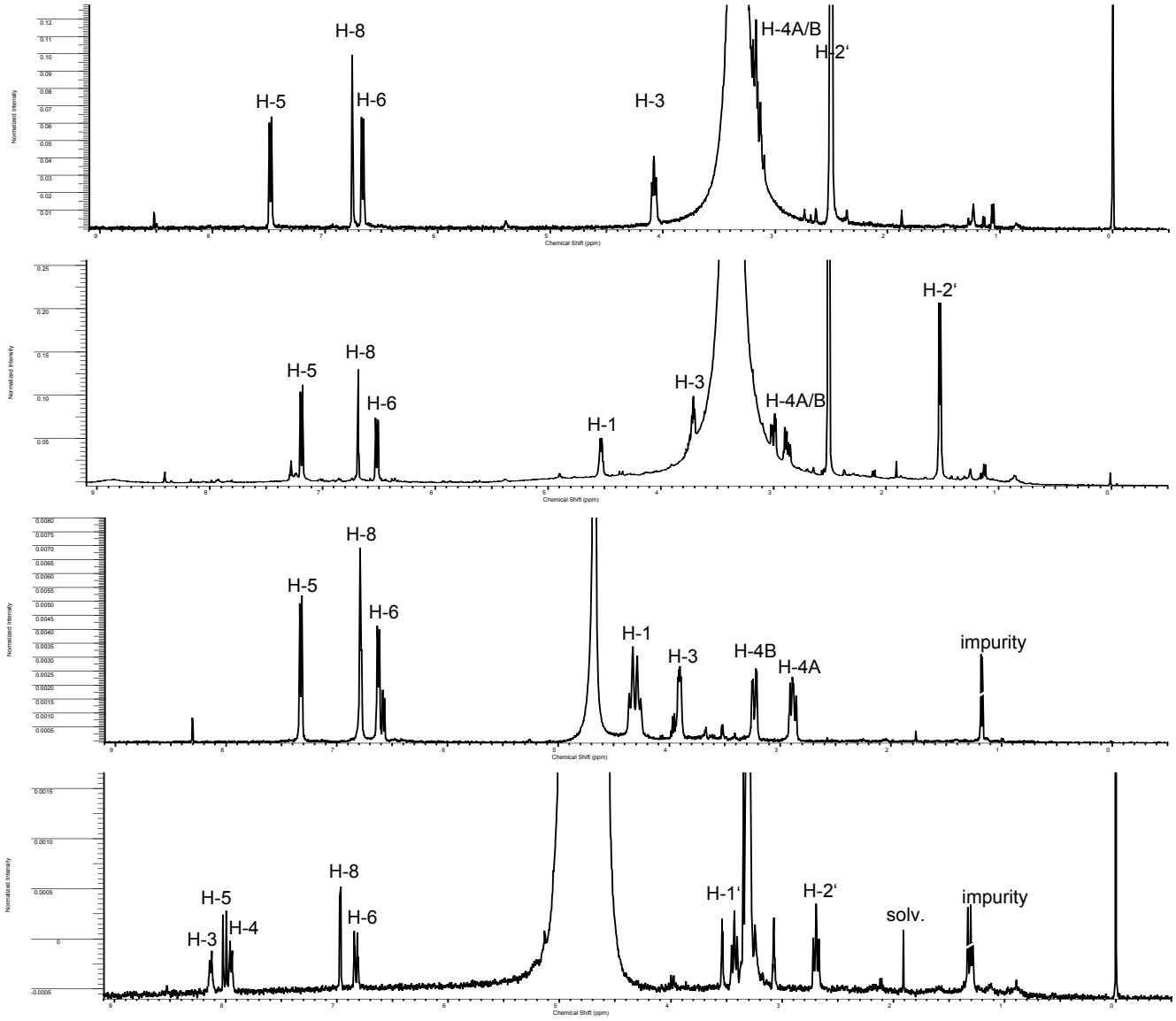
Brunnein A occurs only in section Olivaceoumbrini within the species *H. pustulatus, H. olivaceoalbus,* (*H. persoonii*), *H. agathosmus, H. latitabundus, H.hyacinthinus,* (not in *H. marzuolus* !)

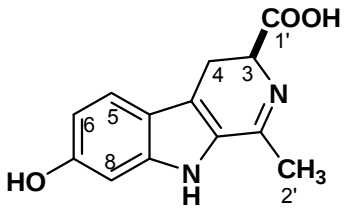
→ **chemotaxonomical marker for this section, comparable to taxonomical cassification based on morphological data**



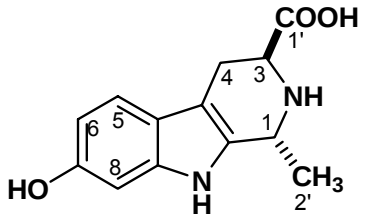
Axel Teichert, Â., Schmidt, J., Kuhnt, C., Huth, M., Porzel, A., Wessjohann, L., Arnold, N. (2008) - Determination of β -carboline alkaloids in fruiting bodies of Hygrophorus spp. by liquid chromatography/electrospray ionisation tandem mass spectrometry. Phytochem. Analysis., in press.

Brunnein A – D: ^{1}H NMR

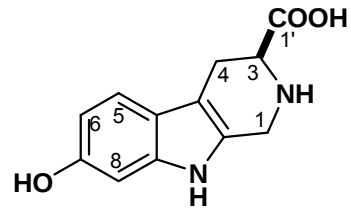




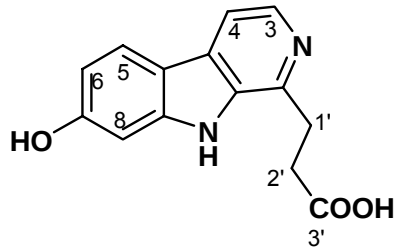
Brunnein A, DMSO-d_6



Brunnein B, DMSO-d_6



Brunnein C, D_2O

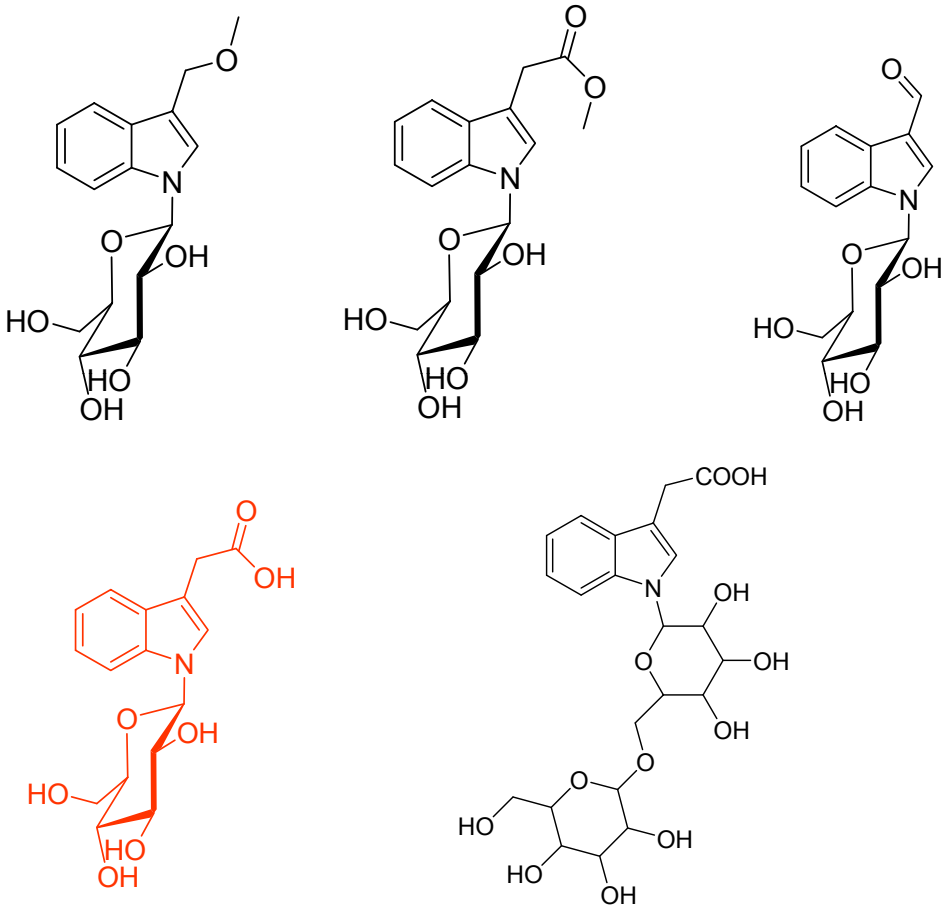


Brunnein D, CD_3OD

β-Carboline Alkaloids from *Cortinarius brunneus*

Cortinarius brunneus

Cortinarius brunneus (365 nm)

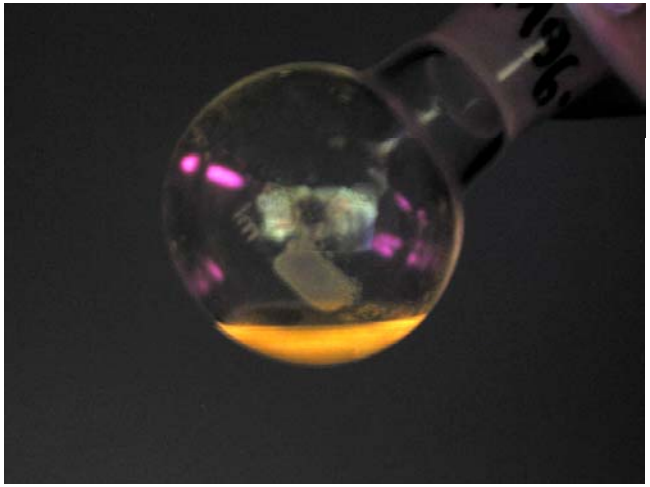


Teichert, A., Schmidt, J., Porzel, A., Arnold, N., Wessjohann, L. (2007) - Brunneins A – C, *β*-carboline alkaloids from *Cortinarius brunneus* (Basidiomycetes) . *J. Nat. Prod*. 70(9), 1529-1531.

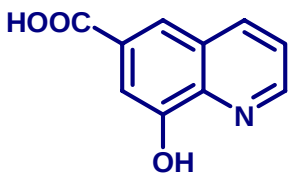
Teichert, A., Schmidt, J., Porzel, A., Arnold, N., Wessjohann, L. (2008) - *N*-Glucosyl Indole Derivatives from *Cortinarius brunneus* (Basidiomycetes). *Chem. Biodiv*. 5, 664-669.

(Iso-) Quinoline Alkaloids from *Cortinarius subtortus*

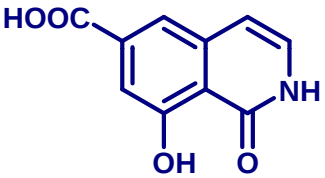
Cortinarius subtortus



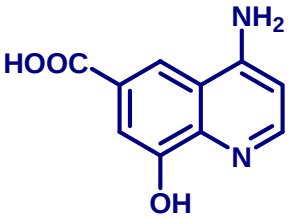
Crude extractc



8-Hydroxyquinoline-6-carboxylic acid



8-Hydroxy-1-oxo-dihydroisoquinoline-6-carboxylic acid

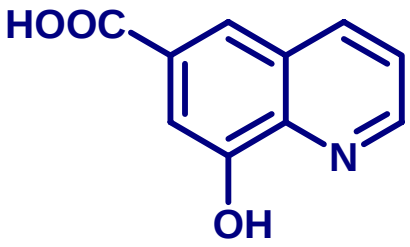


4-Amino-8-hydroxyquinoline-6-carboxylic acid

Teichert, A., Schmidt, J., Porzel, A., Arnold, N., Wessjohann, L. (2008) - (Iso-) Quinoline Alkaloids from *Cortinarius subtortus J. Nat. Prod.*, in press.

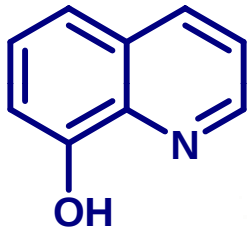
(Iso-) Quinoline alkaloids – similarities!

secondary metabolite from fungi:



8-Hydroxyquinoline-6-carboxylic acid

synthetic compound

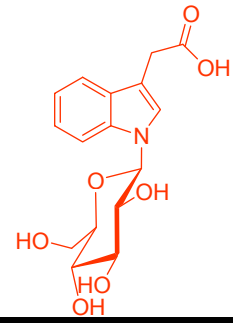


8-Hydroxyquinoline

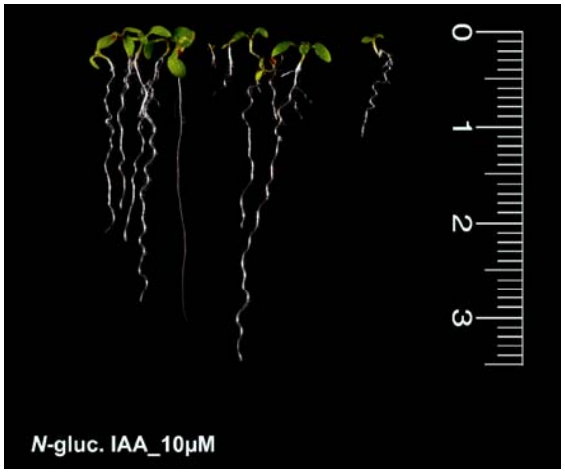
Plant fungizide

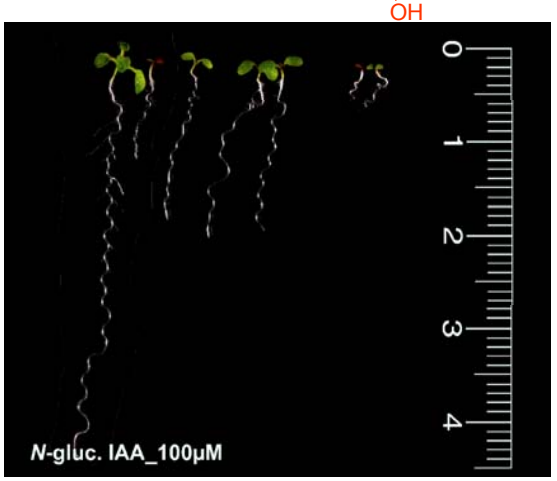
Desinfectants

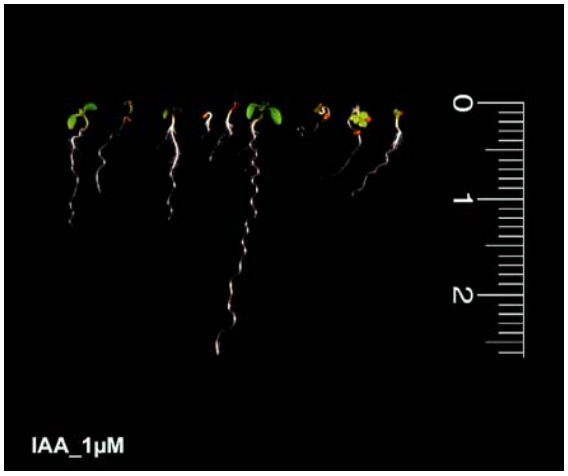
Bioactivity of *N*-Glucosyl-(1*H*-indol-3-yl)-acetic acid

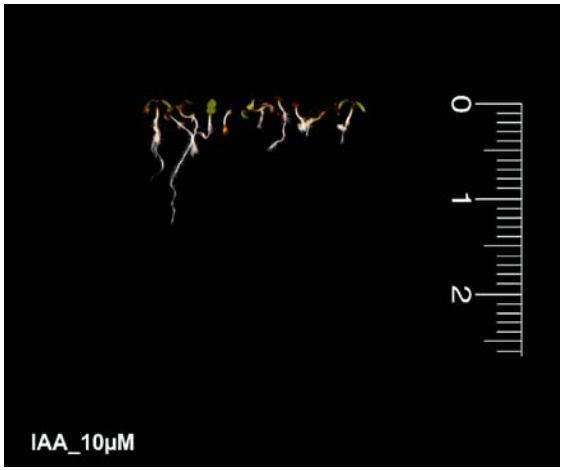


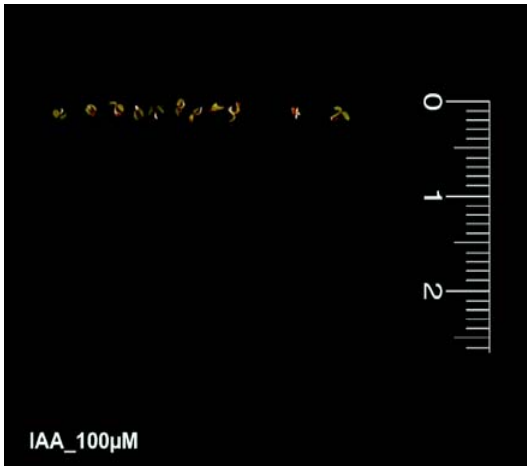
Arabidopsis thaliana tap root elongation assay (10 dpi)



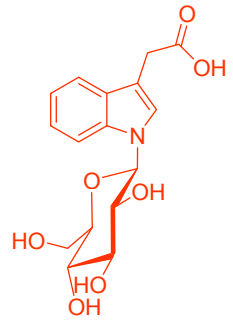




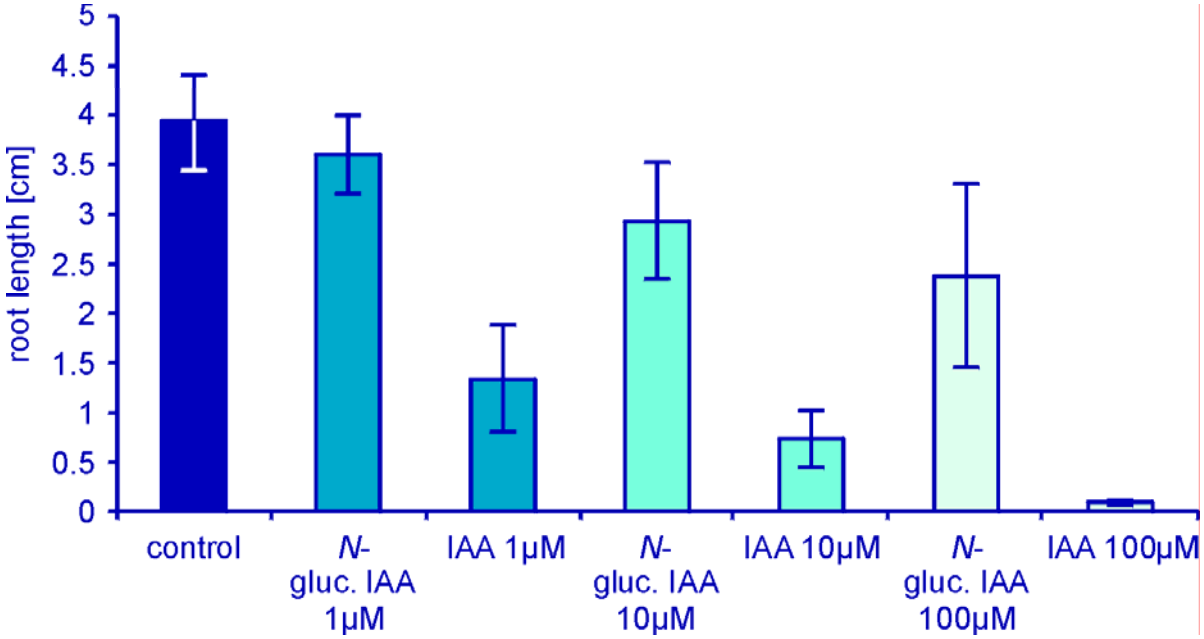




Bioactivity of *N*-Glucosyl-(1*H*-indol-3-yl)-acetic acid



Arabidopsis thaliana tap root elongation assay (10 dpi)

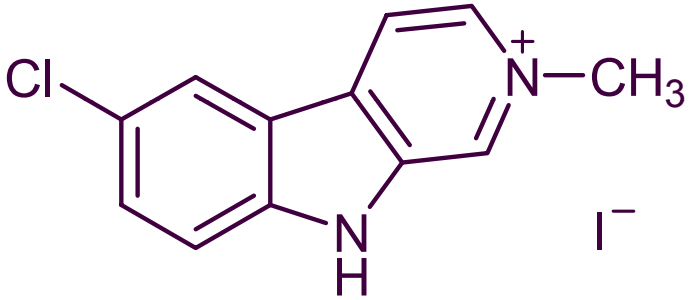


→ no auxin like activity of *N*-gluc IAA

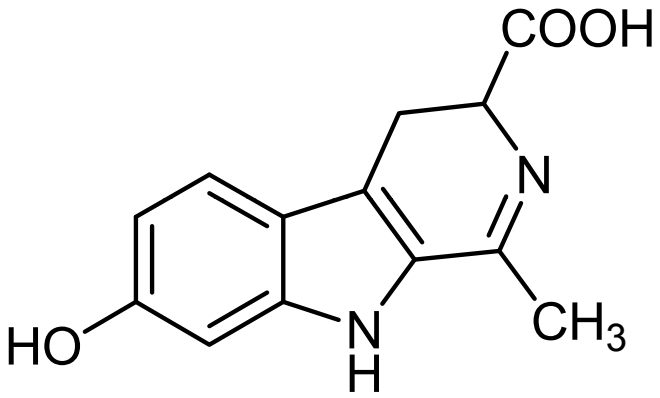
→ possible: inactive storage or transport conjugate?

Bioactivity of Brunnein A

structurally similar to nostocarboline[1], a potent AChE inhibitor



Nostocarboline

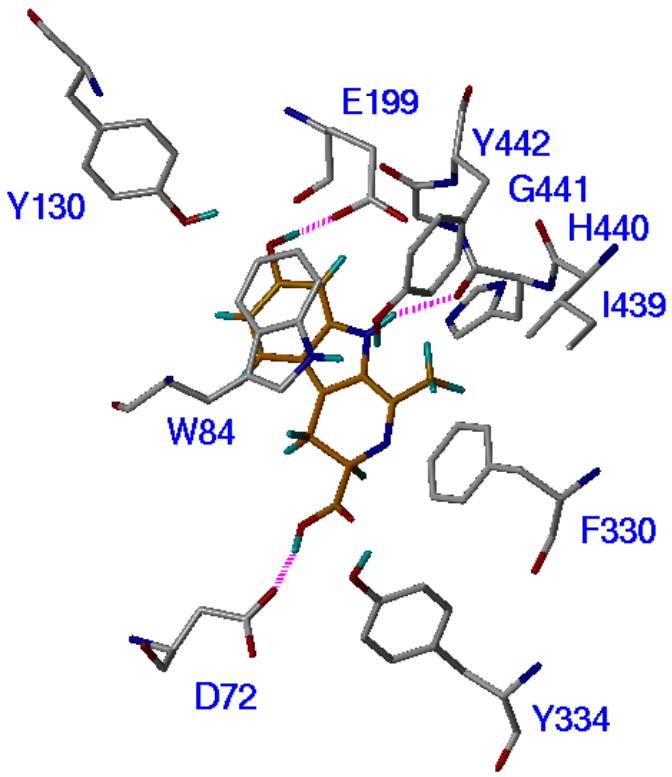


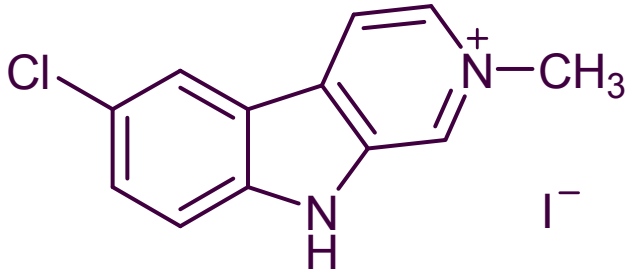
Brunnein A

[1]Paul G. Becher, Julien Beuchat, Karl Gademann, Friedrich Jüttner, 2005 - Nostocarboline: Isolation and Synthesis of a New Cholinesterase Inhibitor from*Nostoc* 78-12A, J. Nat. Prod., 68, 1793-1795.

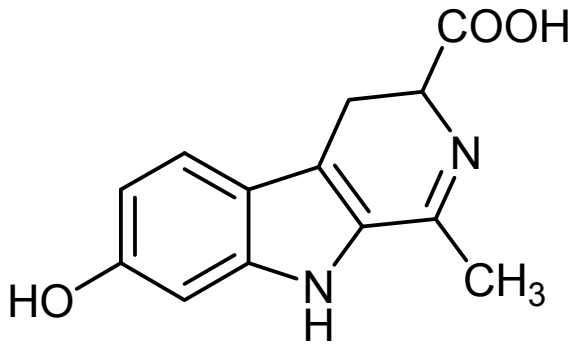
In silico docking studies to AChE

Active site





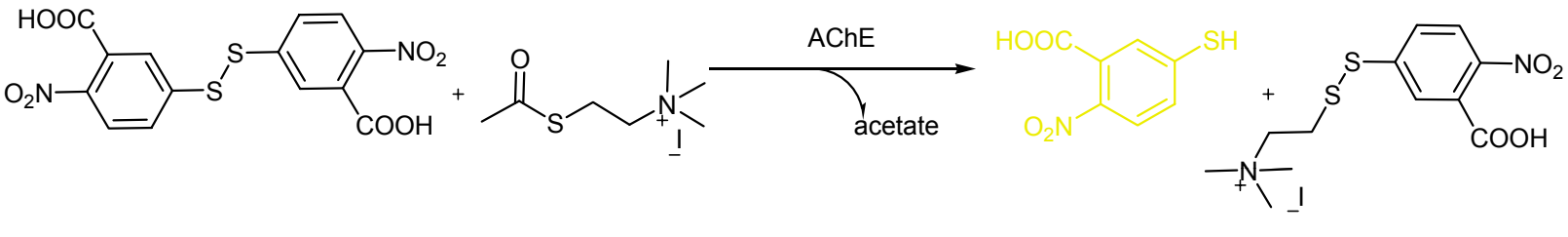
Nostocarboline



Brunnein A

score of Brunnein A > Nostocarboline: H-H bridge

in vitro AChE inhibition assay using Ellman‘s method



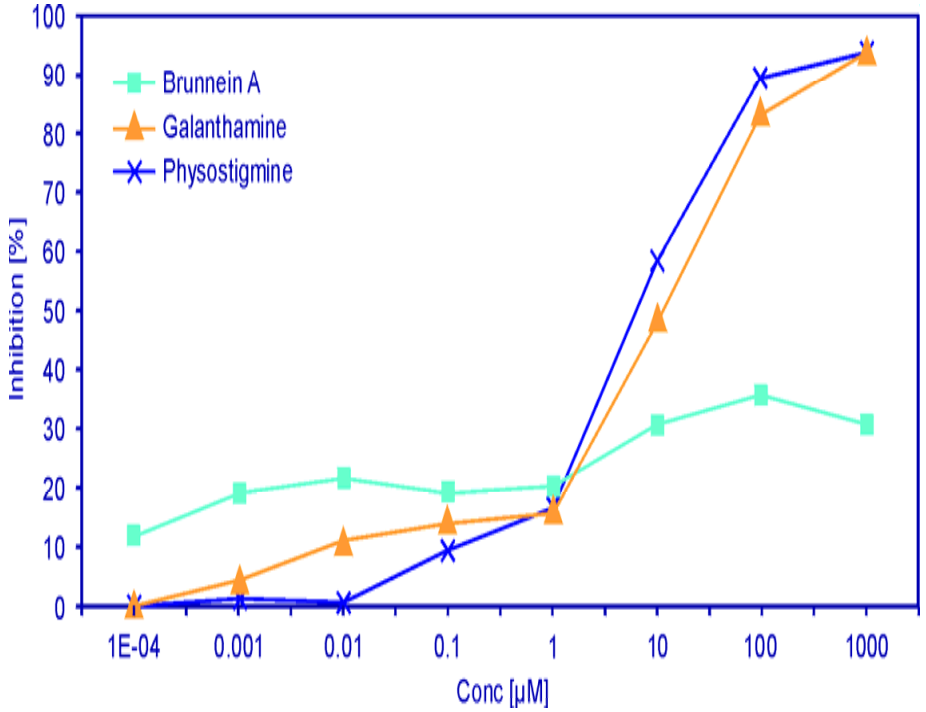
5.5'-dithio-bis(2-nitrobenzoic acid)
DTNB

acetylthiocholine

thionitrobenzoate
TNB

[1] Schott et al., 2006, 6-hydroxy- and 6-methoxy-β-carbolines as acetyl- and butyrylcholinesterase inhibitors, *Bioorg. Med. Chem. Lett*. 12, 5840-5843
[2] Gearhart et al., 2002, Phenylethanolamine N-methyltransferase has β-carboline 2N-methyltransferase activity..., *Neurochem. Int.* 40, 611-620

in vitro AChE inhibition assay using Ellman‘s method



AChE from bovine erythrocytes

- only marginal inhibition effects

- literature: • carbolinium salts best inhibitors, *i.a.* N-Me derivatives[1]
  • β-carboline 2N-methyltransferase in human brain[2]
- pro drug hypothesis → tert. carbolines as prodrugs (lipophil - transport)?
  → metabol. in active carbolinium salts (hydrophil – brain locked)?

[1] Schott et al., 2006, 6-hydroxy- and 6-methoxy-β-carbolines as acetyl- and butyrylcholinesterase inhibitors, *Bioorg. Med. Chem. Lett*. 12, 5840-5843
[2] Gearhart et al., 2002, Phenylethanolamine N-methyltransferase has β-carboline 2N-methyltransferase activity..., *Neurochem. Int.* 40, 611-620

Antifungal assay

Cladosporium cucumerinum (scab or gummosis)

Advantage:

+ good initial test system

+ simply and cheap

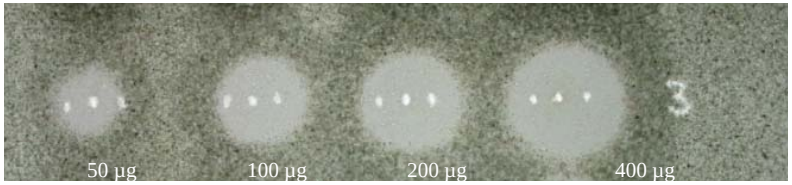
+ results direct observable

Disadvantage:

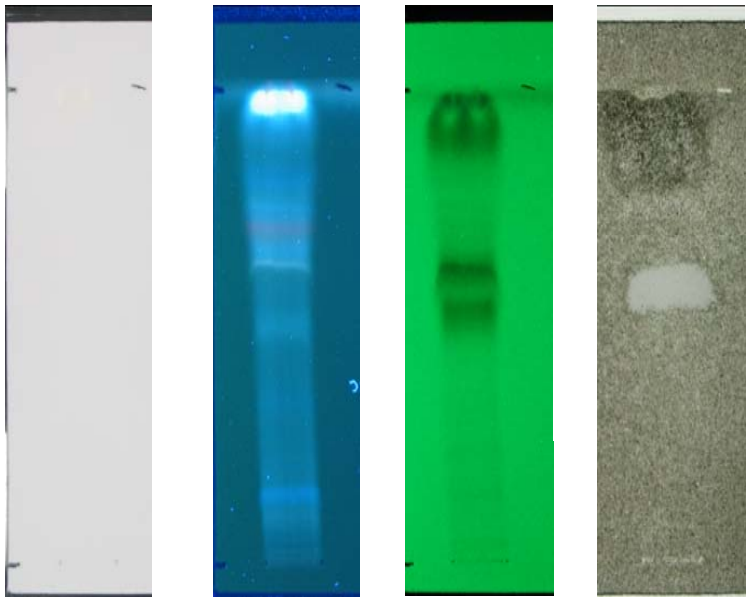
- no information about the concentration of the active compounds
- diffusion of the compounds on the TLC layer
- results not or hardly comparable with other fungicidal assays

Gottstein, D., Gross, D., Lehmann, H. (1982) – Mikrobiotest mit *Cladosporium cucumerinum* Ell. et Arth. zum Nachweis funitoxischer Verbindungen auf Dünnschichtplatten. Arch. Phytopathol. Pfl. 20, 111 - 116

Direct Bioautography on TLC



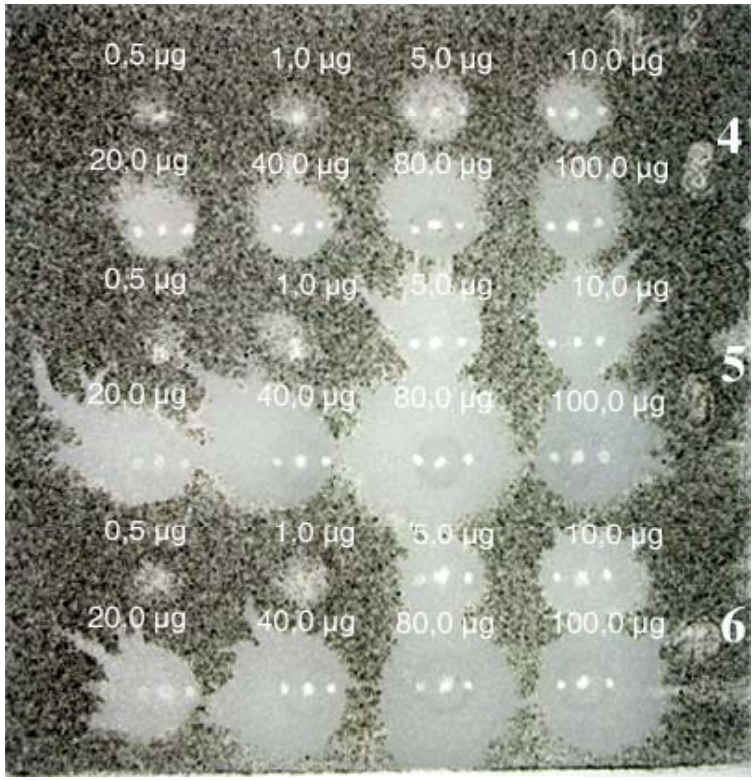
EtOAc crude extract *H. eburneus*

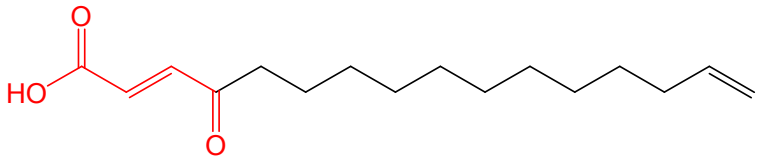


daylight | 365 nm | 254 nm | incubation

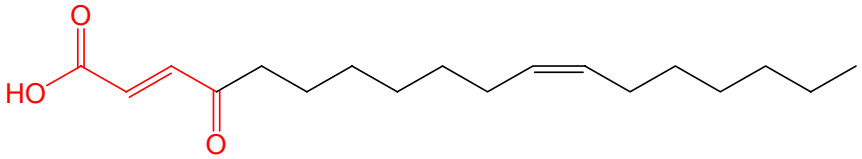
EtOAc crude extract developed on TLC plates

Antifungal activity of 4-Oxo-2-alkenoic fatty acid

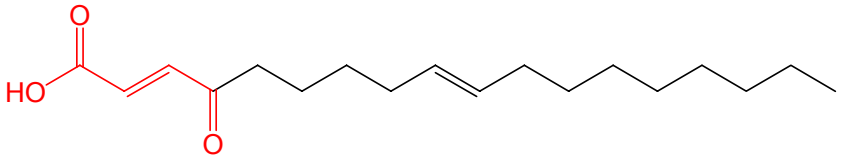




(*E*)-4-oxohexadeca-2,15-dienoic acid

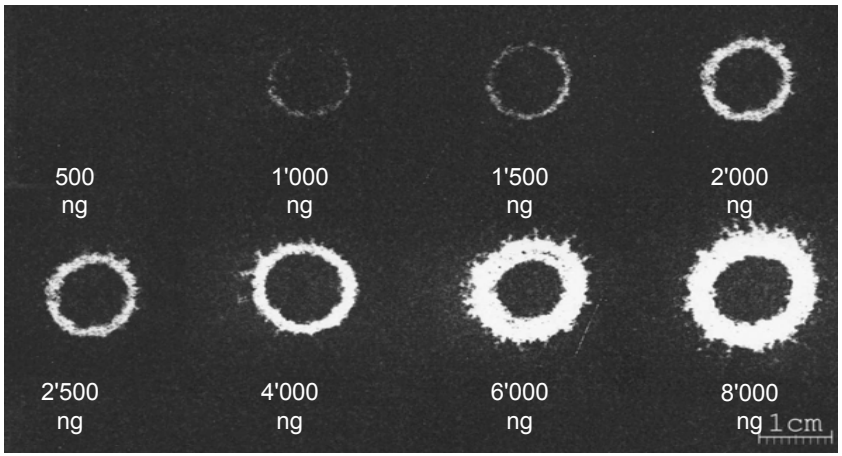


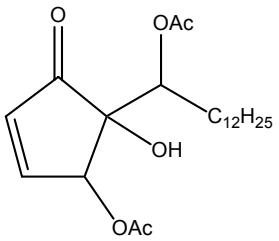
(2*E*,11*Z*)-4-oxooctadeca-2,11-dienoic acid



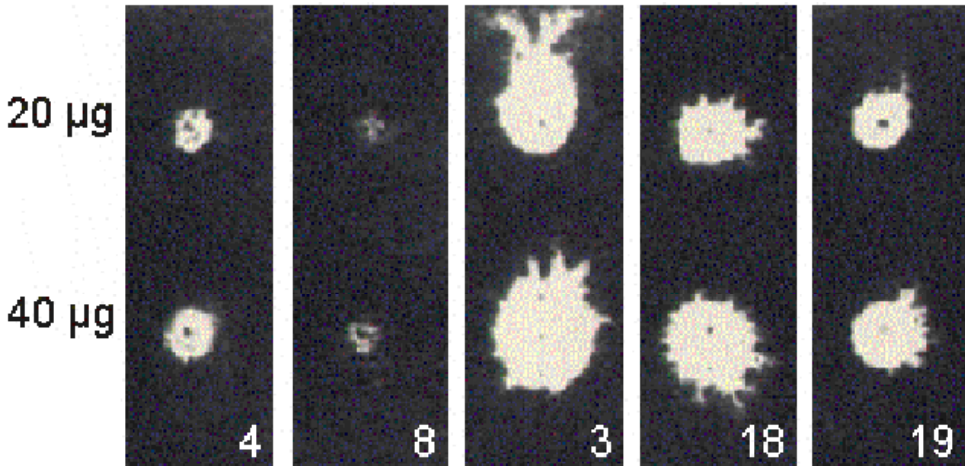
(2*E*,9*E*)-4-oxooctadeca-2,9-dienoic acid

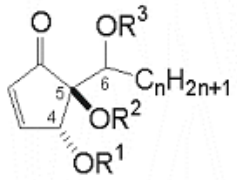
Antifungal activity of Hygrophorones



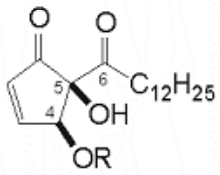


Hygrophoron A





	R^1	R^2	R^3	n	
(1)	H	H	H	12	*Derivat*
(2)	H	H	Ac	12	
(3)	Ac	H	H	12	
(4)	Ac	H	Ac	12	
(5)	Ac	Ac	Ac	12	*Derivat*
(6)	H	H	Ac	14	
(7)	Ac	H	H	14	
(8)	Ac	H	Ac	14	



	R
(18)	Ac
(19)	H

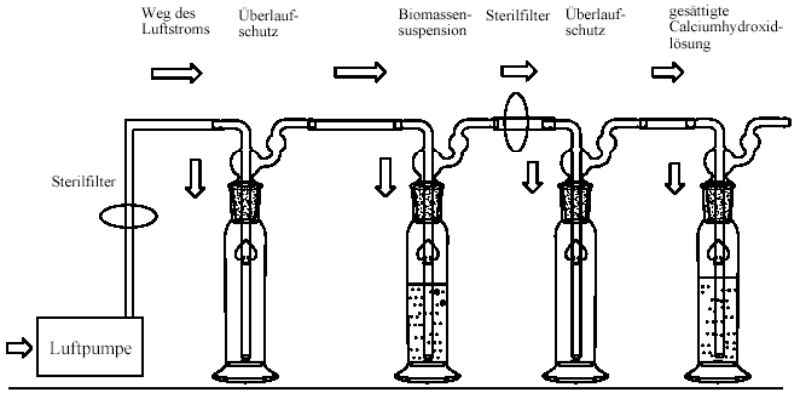
Antialgal activity

Need for ecologically safe Antifowling agents

Testorganisms:
Spirulina laxissima
(blue algae)
Scenedesmus rubescens
(green algae)

TLC with direct bioautography

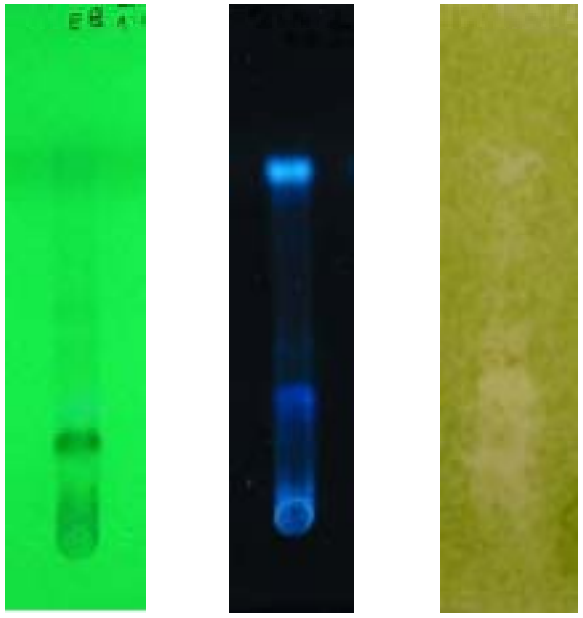
Problem: cultivation and biomass upscale require special equipment and are time consuming:
~ 3-4 weeks upscaling in light chamber and ventilation



Direct Bioautography

50 µg 100 µg 200 µg 400 µg

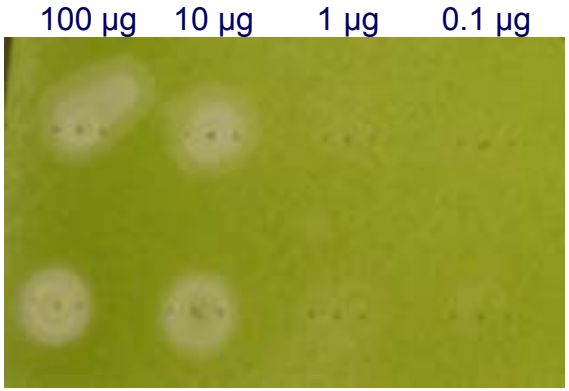
MeOH crude extract *H. eburneus*



254 nm 365 nm incub.

MeOH crude extract developed on TLC plates

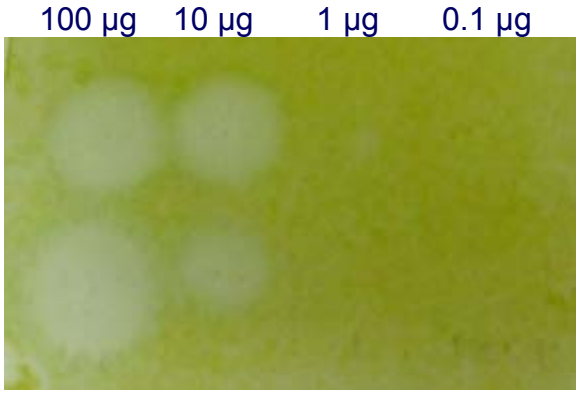
Antialgal activity of Harman and Norharman



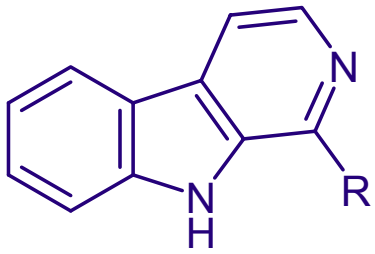
Spirulina laxissima

Norharman

Harmane



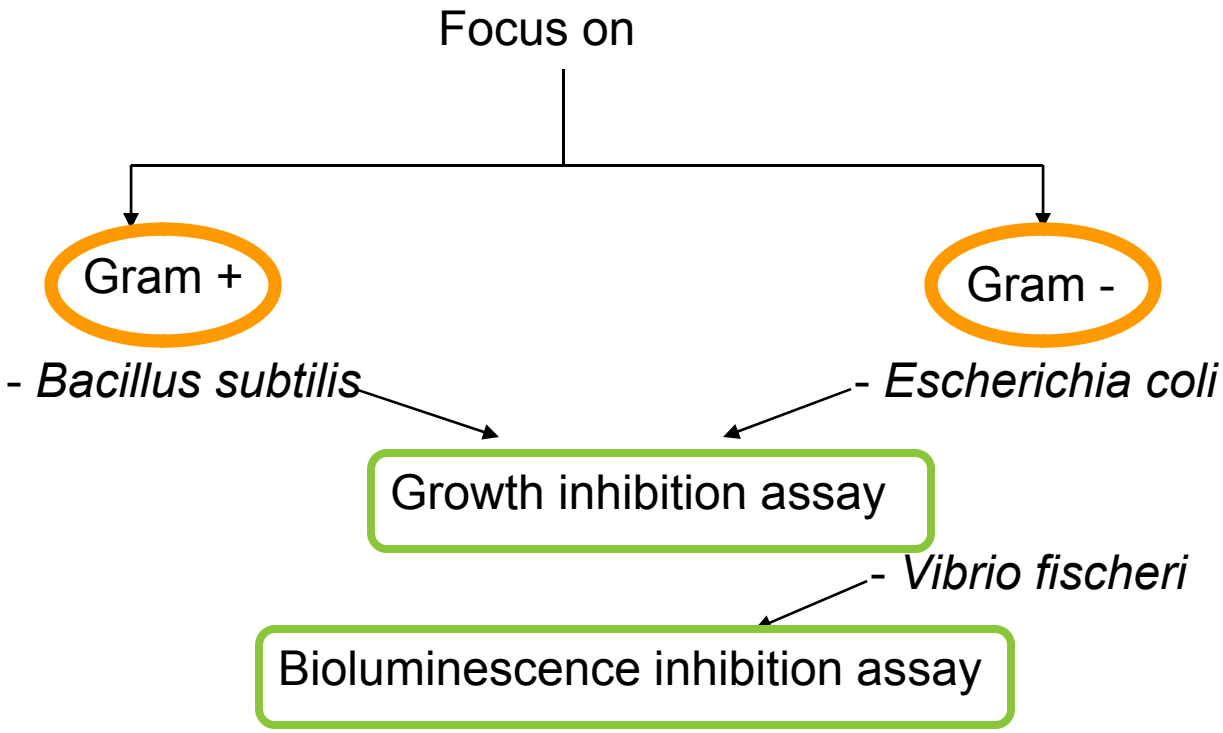
Scenedesmus rubescens



R: H – Norharman

CH_3 – Harman

Antibacterial bioassays



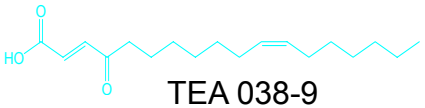
Antibacterial activity: Growth inhibition assay of 4-Oxo-2-alkenoic fatty acid

E. coli (-)

Streptomycin

Amikacin

Rifampicin



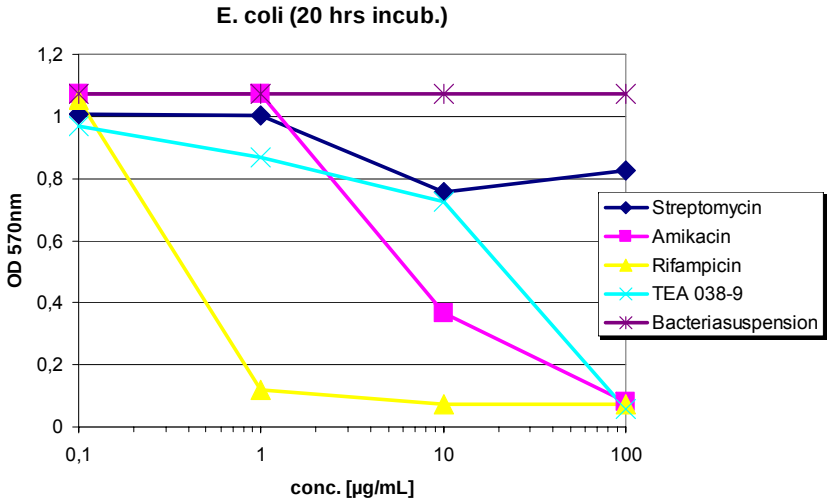
TEA 038-9

a) visual evaluation

b) optical evaluation

	100	10	1	0,1 g/mL
Streptomycin	-	-	-	-
Amikacin	++	+	-	-
Rifampicin	++	++	++	-
TEA 038-9	++	+	-	-

++ : strong inhibition; + : weak inhibition; - : no inhibition



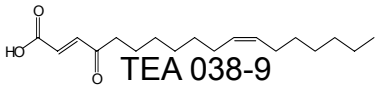
Antibacterial activity: Growth inhibition assay of 4-Oxo-2-alkenoic fatty acid

B. subtilis (+)

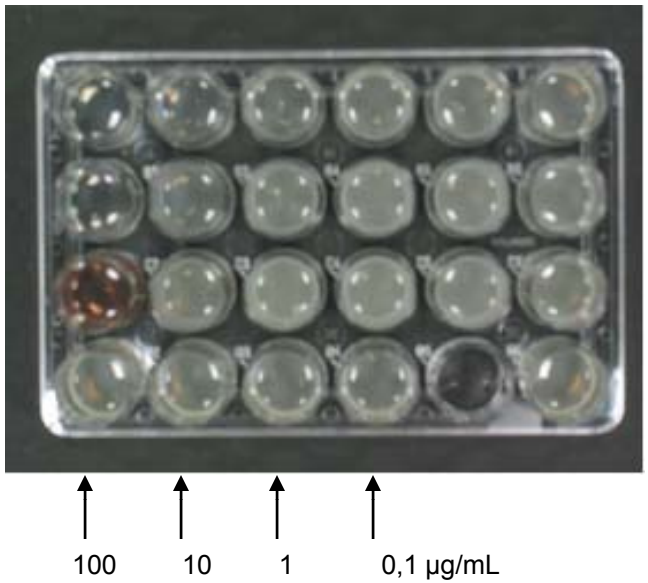
Streptomycin

Amikacin

Rifampicin



TEA 038-9

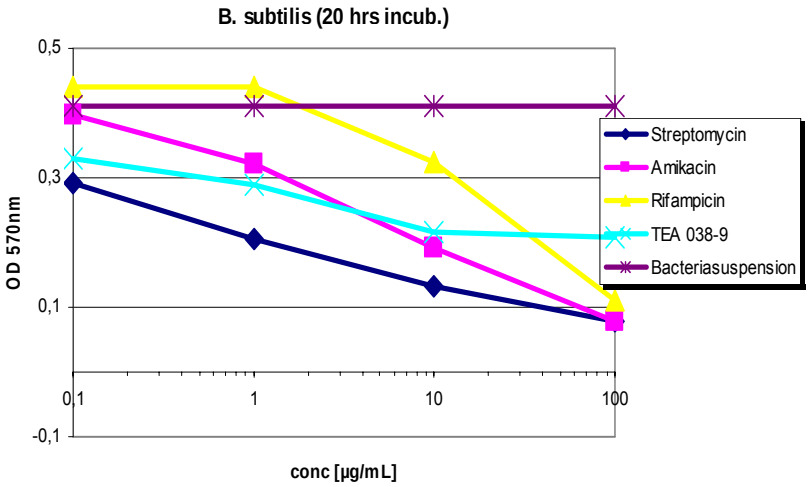


a) visual evaluation

	100	10	1	0,1 g/ml
Streptomycin	++	+	-	-
Amikacin	++	+	-	-
Rifampicin	++	+	-	-
TEA 038-9	+	+	-	-

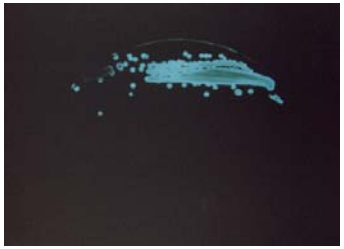
++ : strong inhibition; + : weak inhibition; - : no inhibition

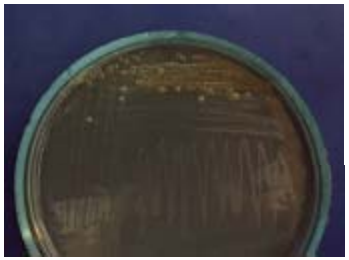
b) optical evaluation

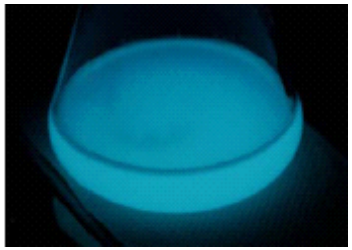


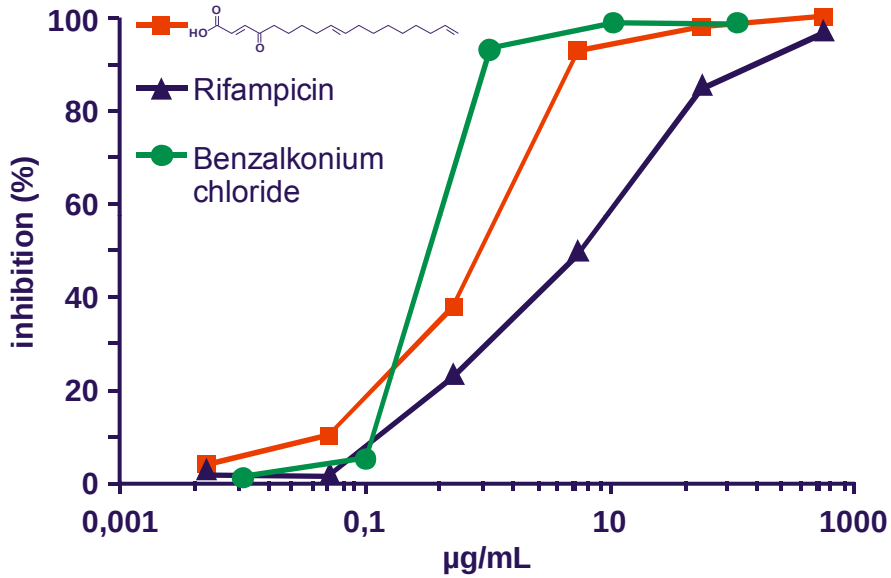
Antibacterial activity: Bioluminescence inhibition assay of 4-Oxo-2-alkenoic fatty acid

Vibrio fischeri (-)



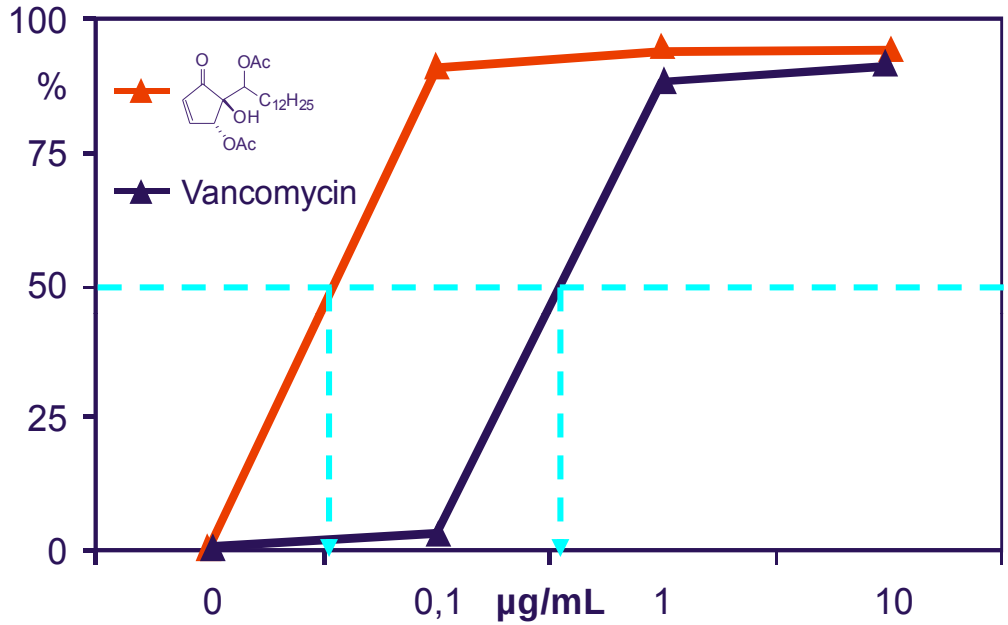






Antibacterial activity: Growth inhibition assay of selected Hygrophorones

methicillin resistant *Staphylococcus aureus* (+)



Anti-Oomycete activity

P. infestans

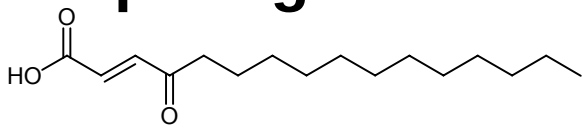
- pathogen of potato blight
  (Irish potato famine 1845-1850)

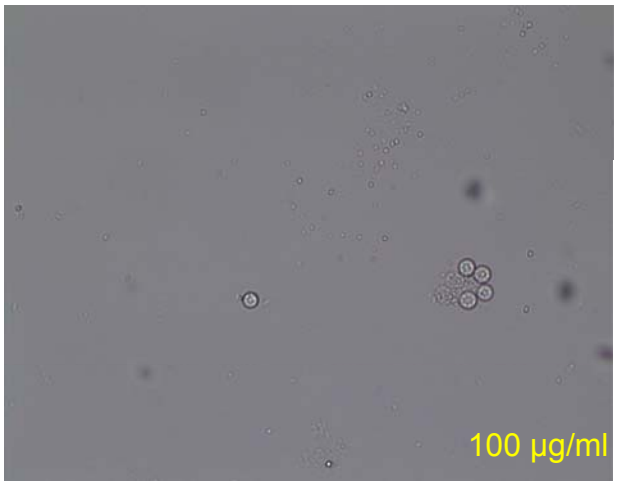
- 2 different bioassays:

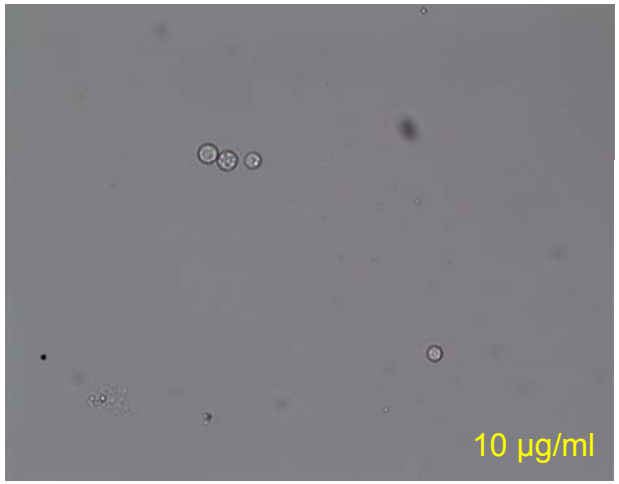
a) spore germination inhibition assay:
   evaluation under microscope

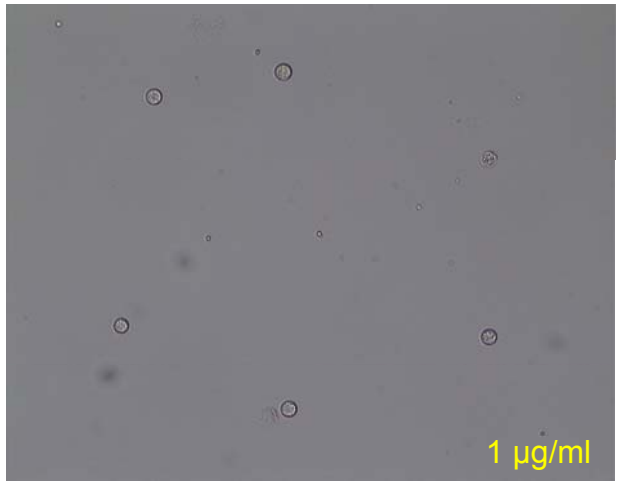
b) mycelial growth inhibition assay:
   evaluation via fluorescence
   measurements using a strain
   expressing GFP as a vital marker

P. infestans spore germination assay

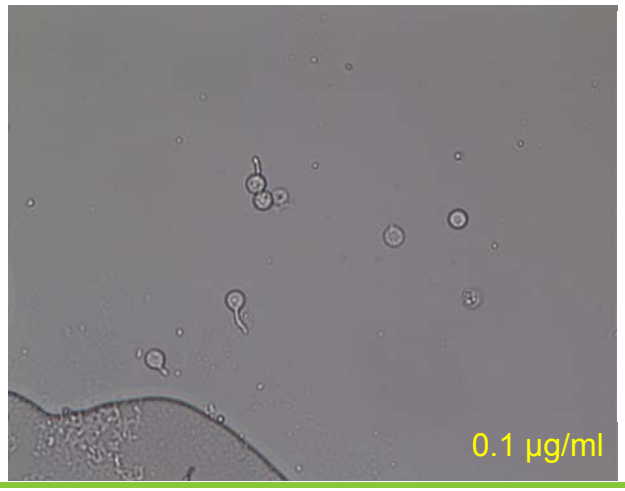


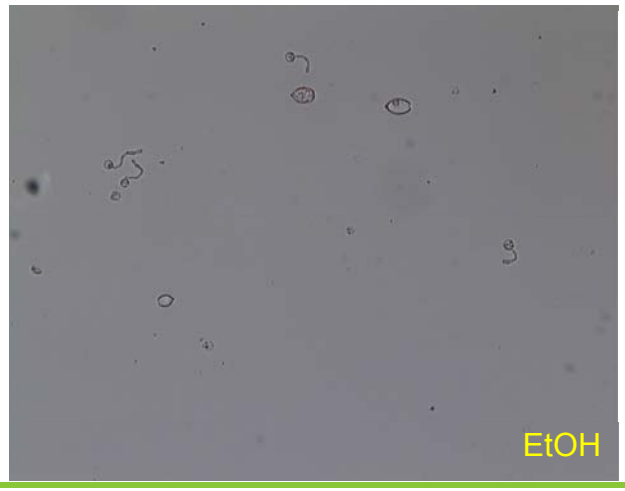




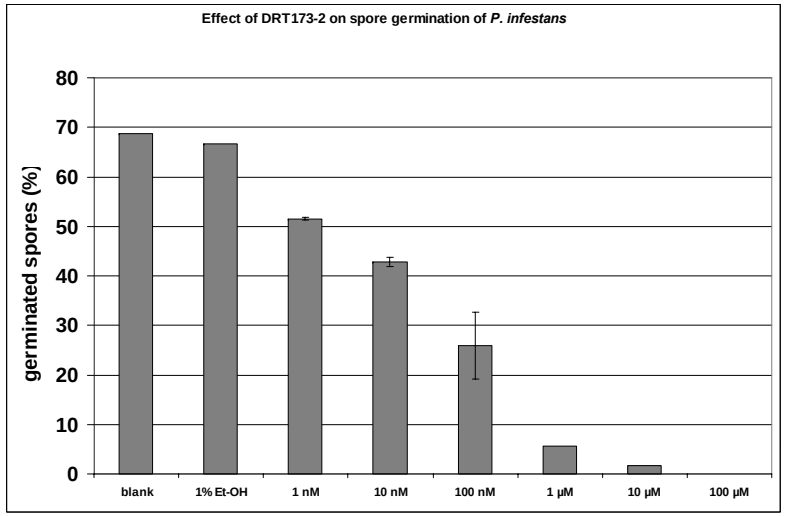


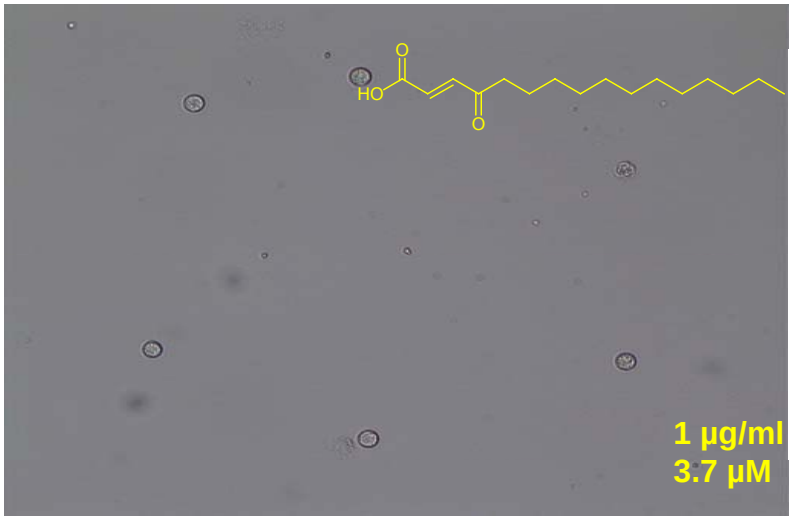
Many bursted spores

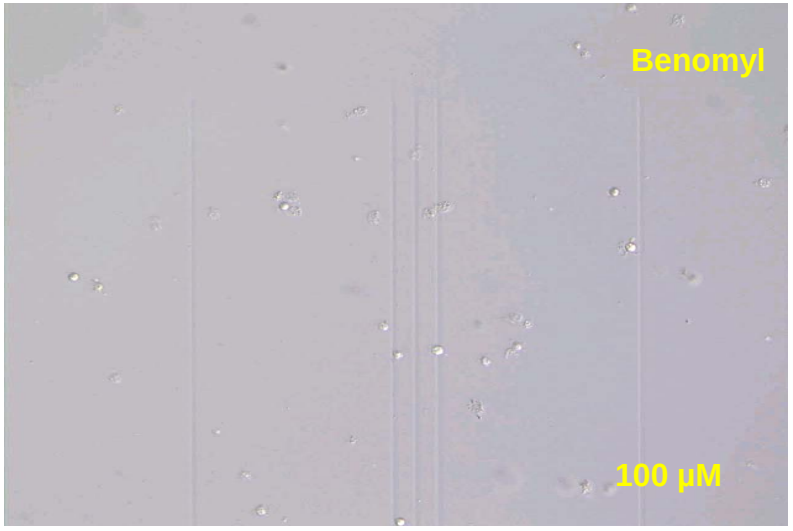


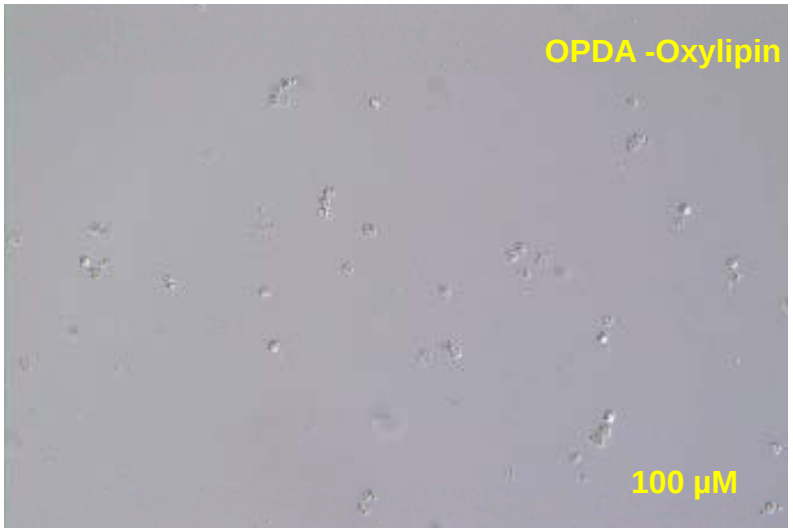


P. infestans spore germination assay

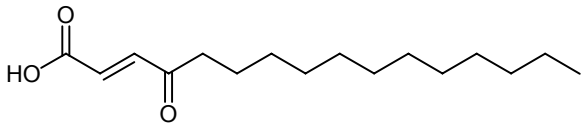


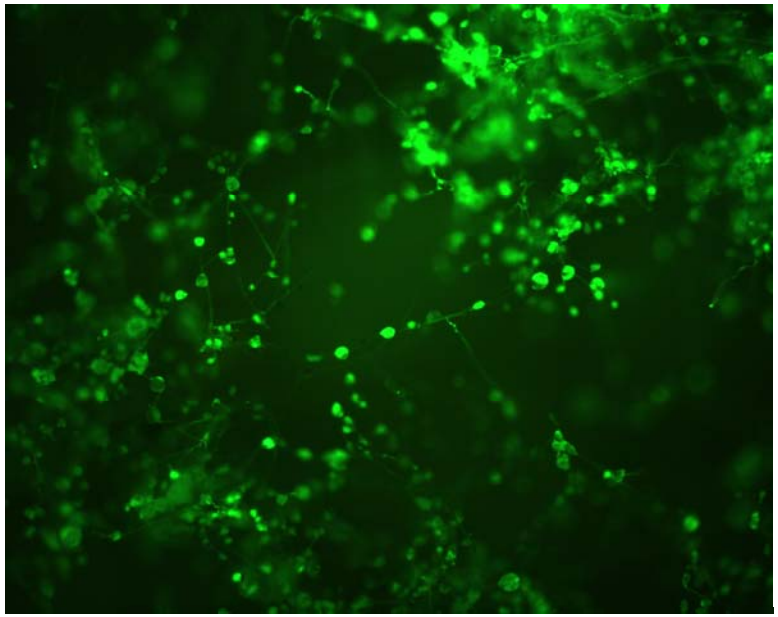






P. infestans mycel growth inhibition assay

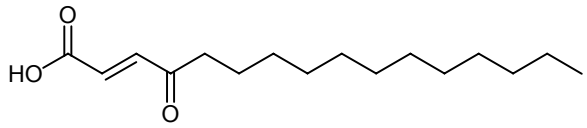


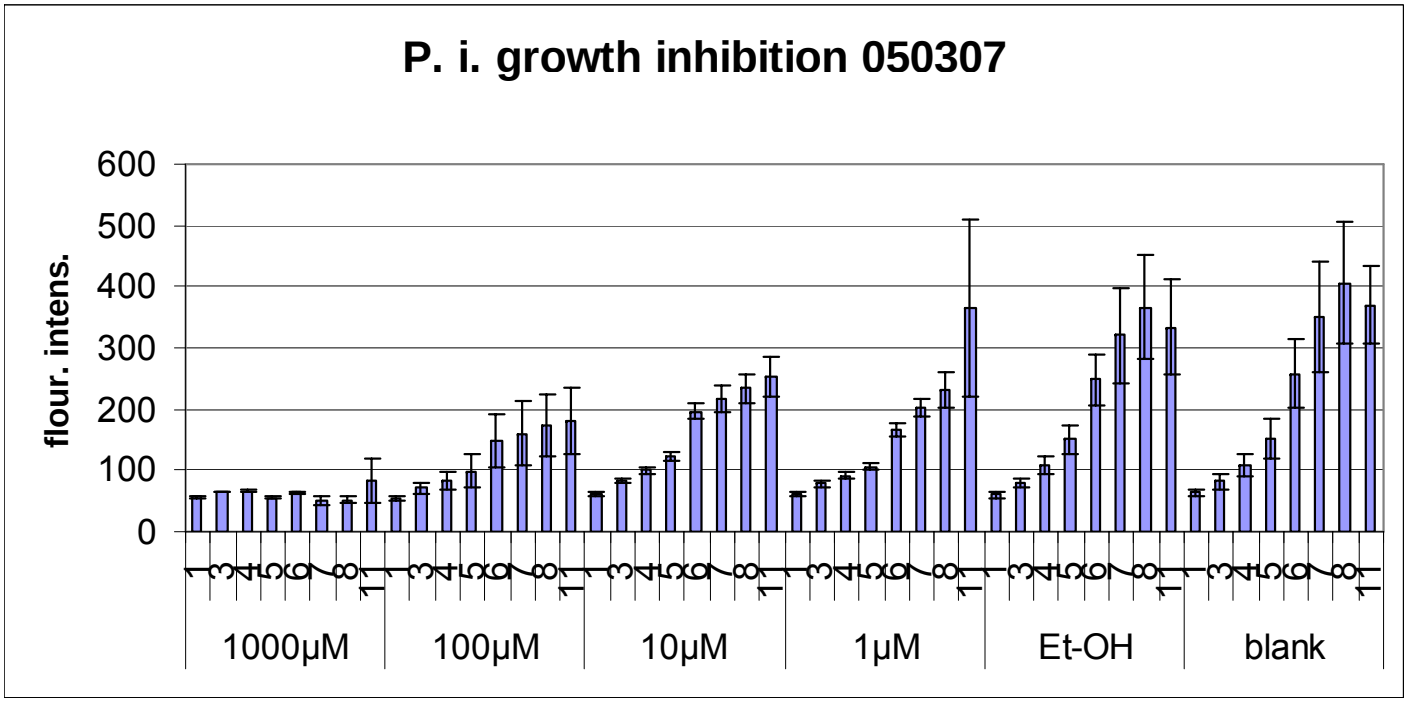


GFP labeled *P. infestans* strain

Visual evaluation after 11 dpi

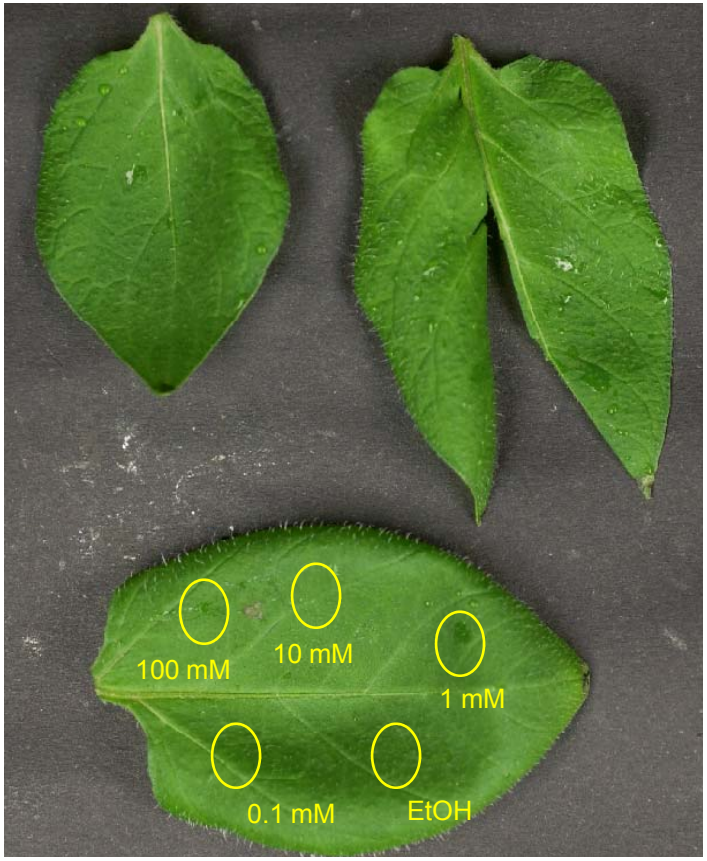
P. infestans mycel growth inhibition assay

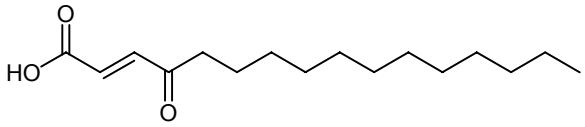




Fluorescence intensity measurements after 11 dpi

P. infestans mycel growth inhibition assay: toxicity for *Solanum tuberosum* leaves





3 dpi, 4 different concentrations

No necrotic effects visible

Conclusion

Secondary metabolites from *Hygrophorus* (and *Cortinarius* ?) act as chemical defense substances

Bacterizidal effects

Hygrophorones: very potent against (+) gram bacteria (MRSA)
Fatty acids: high activity against (–) gram bacteria (*V. fischeri, E. coli*)

Fungizidal effects

Hygrophorones: high activity against *C. cucumerinum*
Fatty acids: high activity against *C. cucumerinum*

Oomycetes

Fatty acids: high activity in spore gemination and mycel growing assay (*P. infestans*)

Algizidal effects

Harman and Norharman: active against *Sp. laxissima, Sc. Rubescens*

AChE-assay

Brunnein A: weak activity

Co - worker

Axel Teichert

Monika Kummer

Dr. Tilo Lübken

Dr. Jürgen Schmidt

Dr. Andrea Porzel

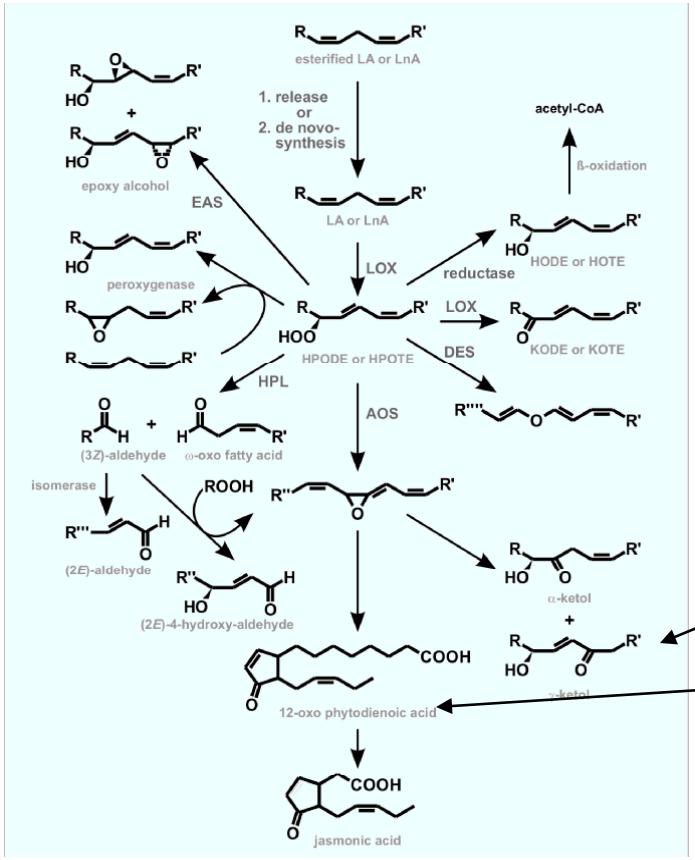
Prof. Dr. L. Wessjohann, H

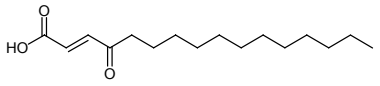
Financial support

DFG, MK LSA

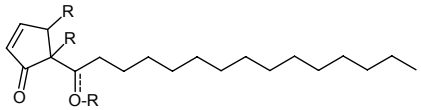
Plant hormones – pathogen defense

→ Similarities in *Hygrophorus* ?!





Oxocrotonate FA ?

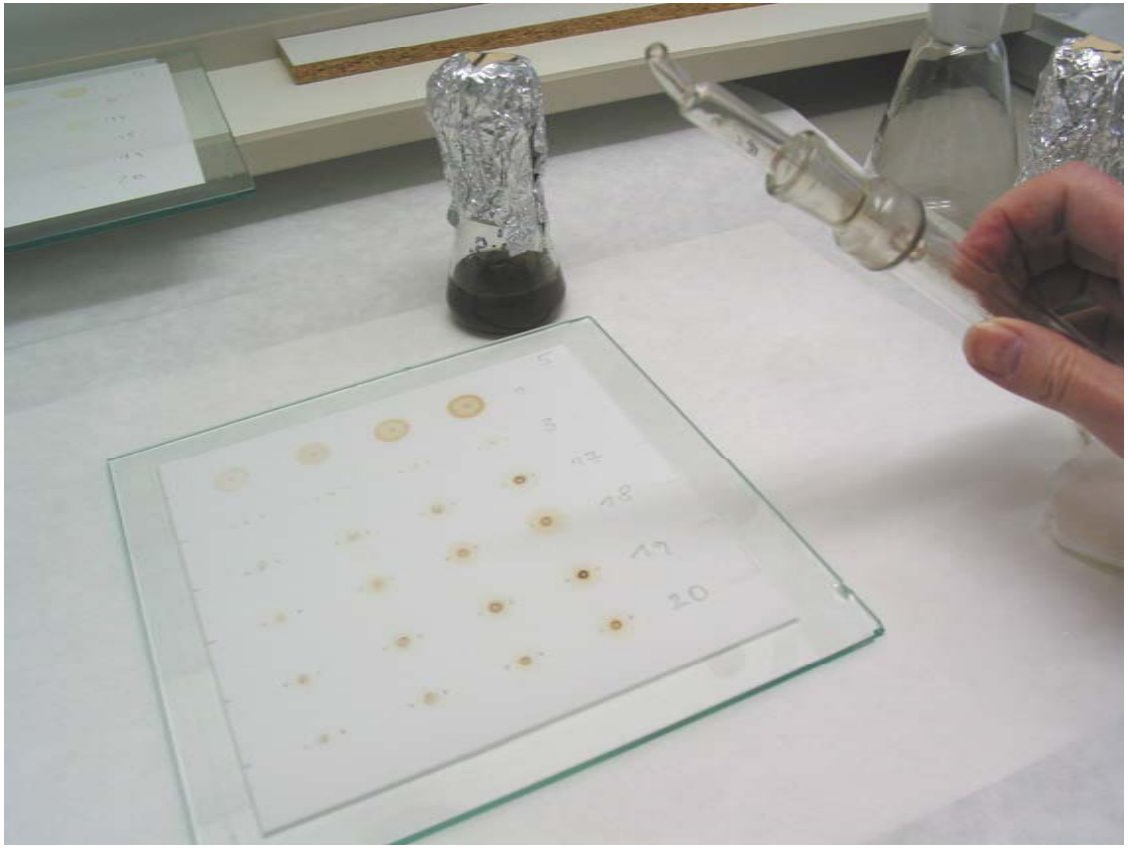


Hygrophorones ?

Plant system –
jasmonic acid pathway induced by pathogens
including lots of antimicrobial oxylipins

Fungi system ?

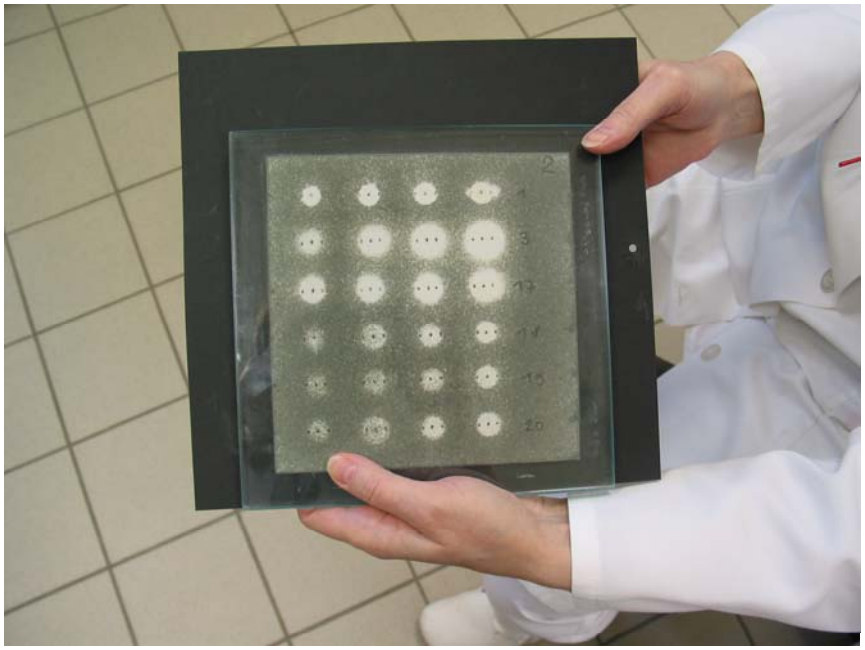
Spraying of the TLC plates with conidiospores of *C. cucumerinum*

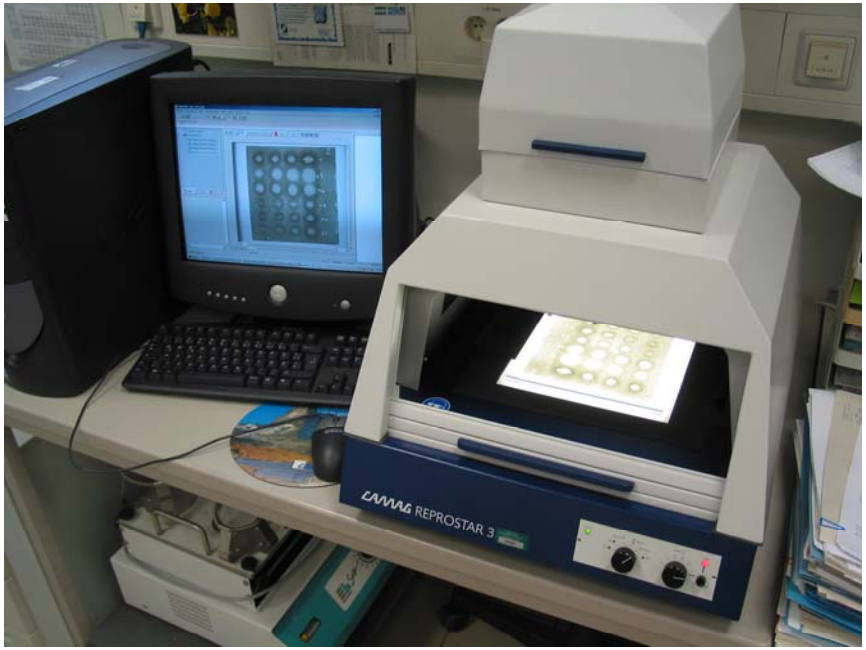


Growing of the coloured mycelia in a moisty atmosphere

(2 days, 25°C)

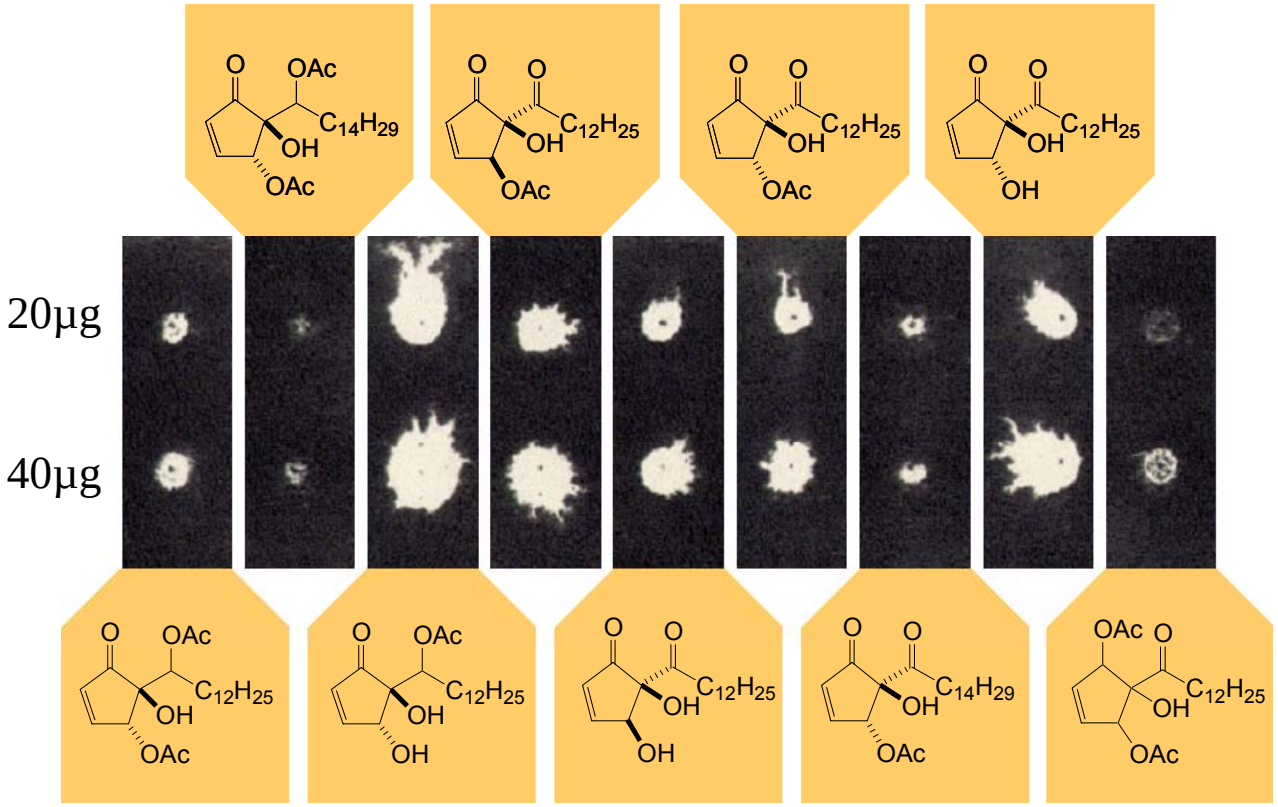
Dokumentation





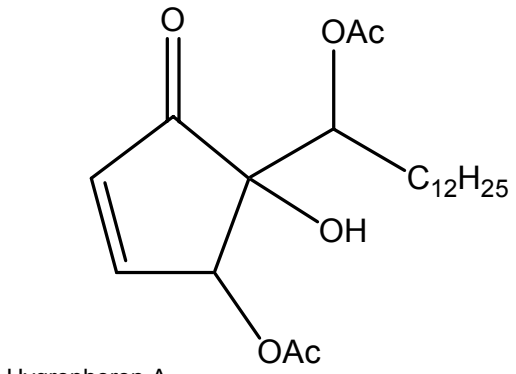
Bioactivity of Hygrophorones A – G

fungicidal activity: *C. cucumerinum*

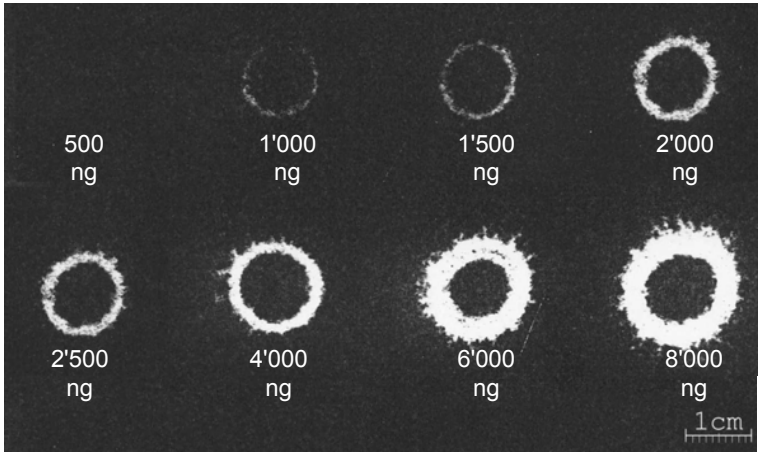


Bioactivity of Hygrophorones

fungicidal activity: *C. cucumerinum*

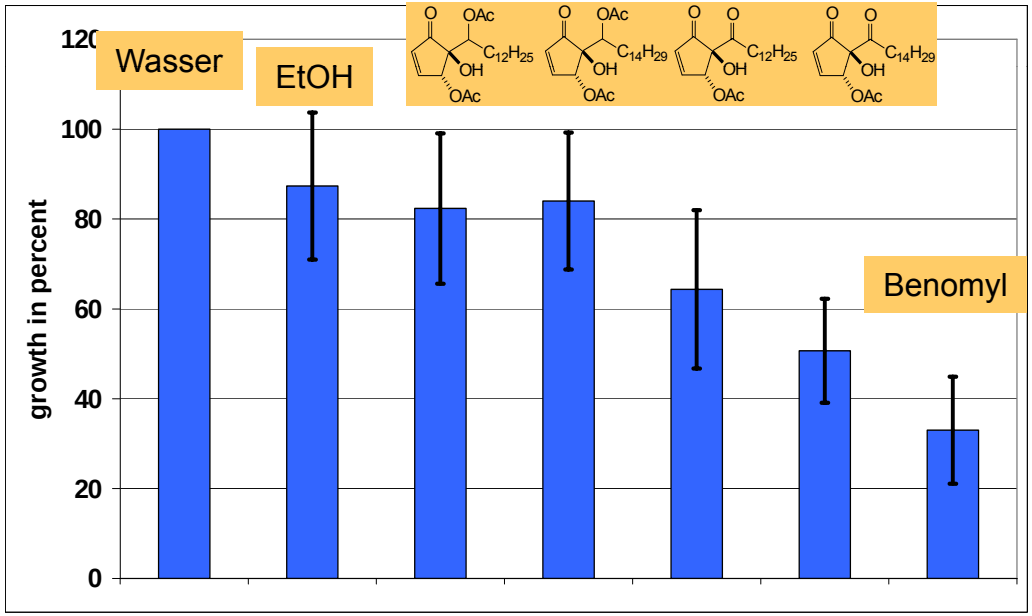


Hygrophoron A



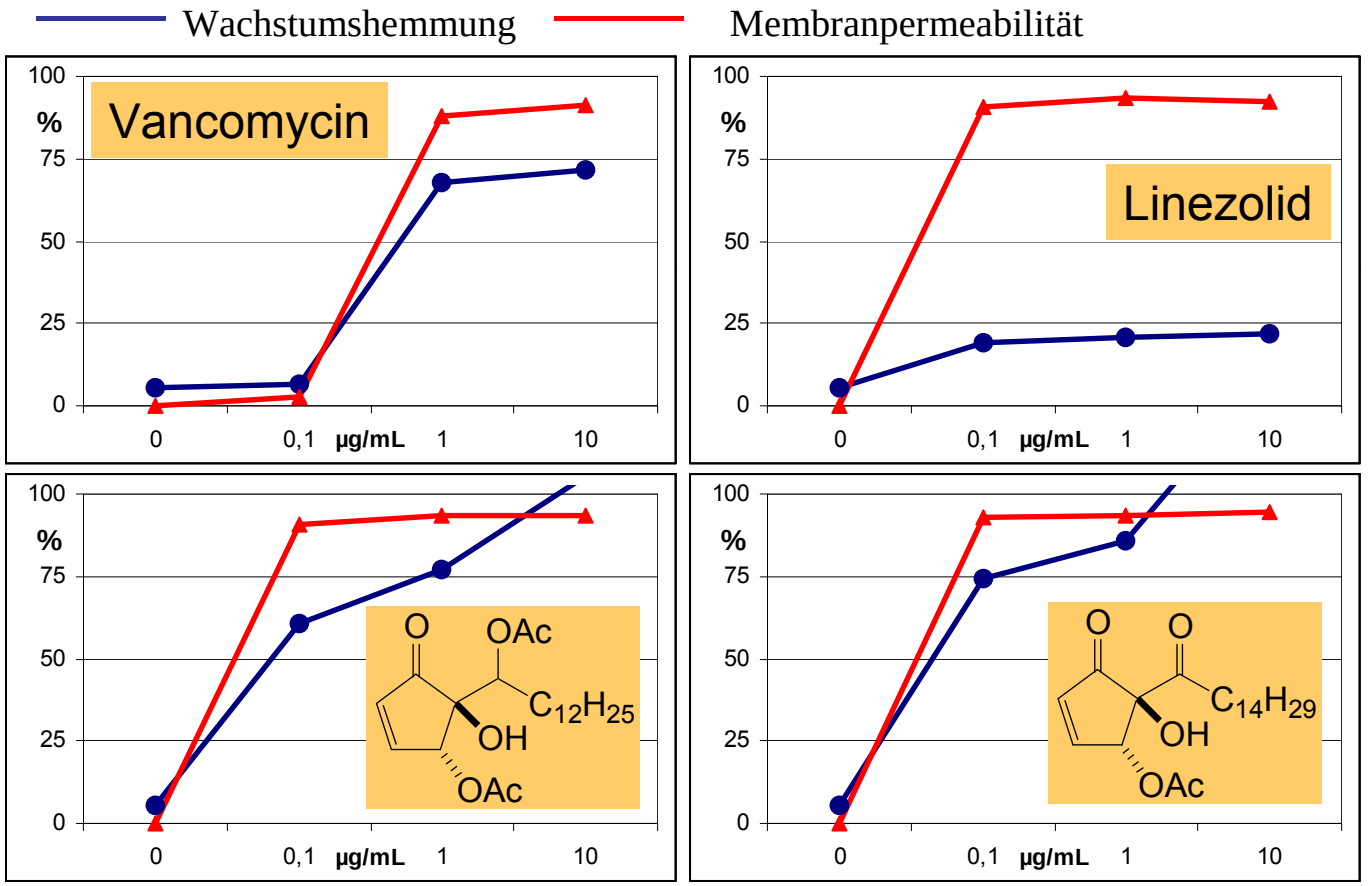
Bioactivity of Hygrophorones

fungicidal activity: *Phytophtora infestans* responsible for potato light



Bioactivity of Hygrophorones

bactericidal activity: MSR *Staphylococcus aureus*



Hygrophorus Sect. Hygrophorus

H. cossus

H. eburneus

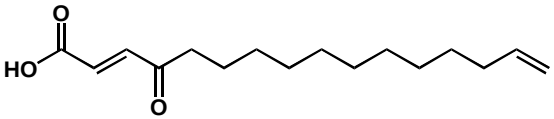
H. discoxanthus

species	***host***	***colour in exsiccate***
H. cossus	*Quercus*	white to ochraceous
H. discoxanthus	*Fagus*	brown to dark brown
H. eburneus	*Fagus*	white to ochraceous
H. picea	*Picea*	white to ochraceous

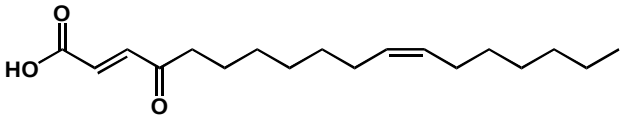
Cossus cossus L.

(= Goat Moth, from the strong 'goaty' odour of the caterpillar)

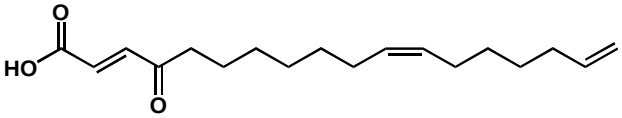
Unusual bioactive fatty acids from *H. eburneus*



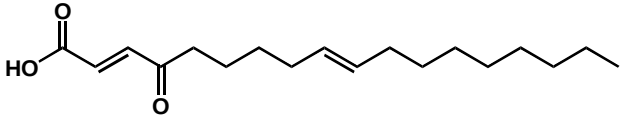
(*E*)-4-oxohexadeca-2,15-dienoic acid (**1**)



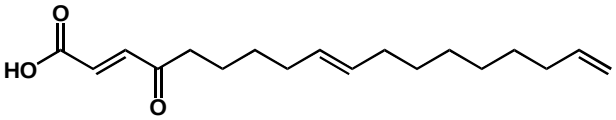
(2*E*,11*Z*)-4-oxooctadeca-2,11-dienoic acid (**5**)



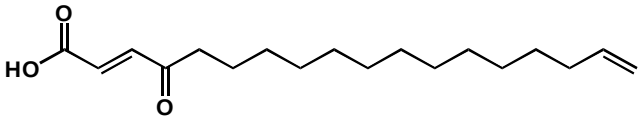
(2*E*,11*Z*)-4-oxooctadeca-2,11,17-trienoic acid (**2**)



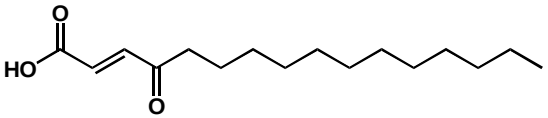
(2*E*,9*E*)-4-oxooctadeca-2,9-dienoic acid (**6**)



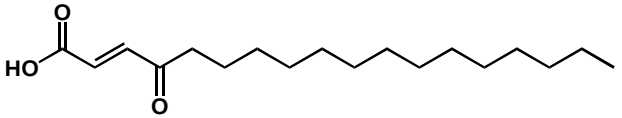
(2*E*,9*E*)-4-oxooctadeca-2,9,17-trienoic acid (**3**)



(*E*)-4-oxooctadeca-2,17-dienoic acid (**7**)



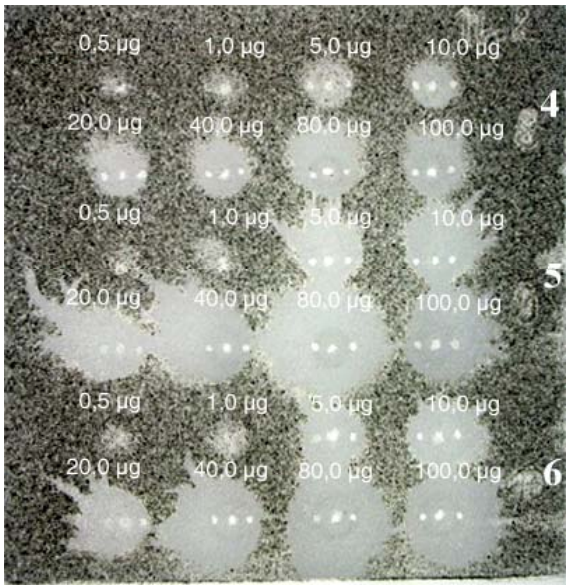
(*E*)-4-oxohexadeca-2-enoic acid (**4**)

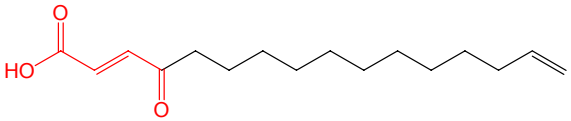


(*E*)-4-oxooctadeca-2-enoic acid (**8**)

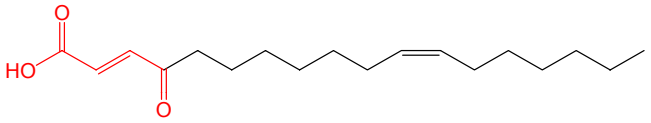
Bioactivity of the isolated fatty acids

fungicidal activity: *C. cucumerinum*

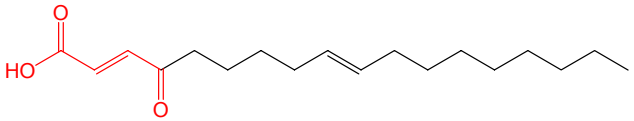




(*E*)-4-oxohexadeca-2,15-dienoic acid



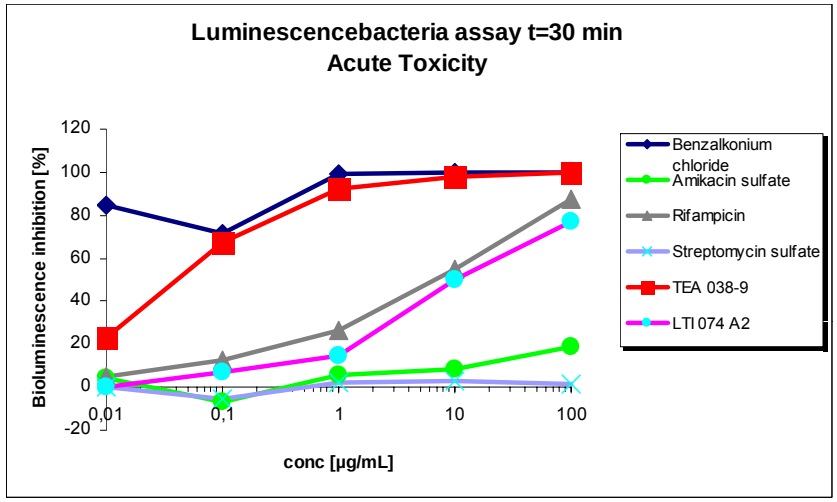
(2*E*,11*Z*)-4-oxooctadeca-2,11-dienoic acid

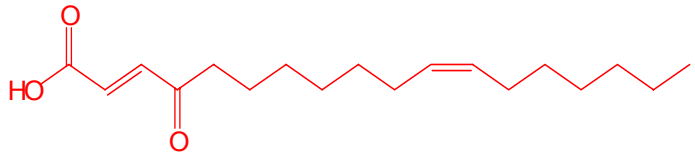


(2*E*,9*E*)-4-oxooctadeca-2,9-dienoic acid

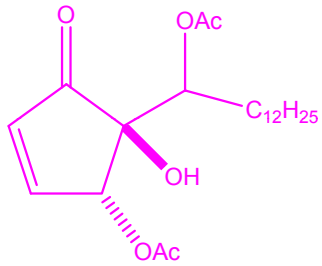
Bioactivity of the isolated fatty acids

Bactericidal activity: *Vibrio fischeri* (-)*:* 30 min assay

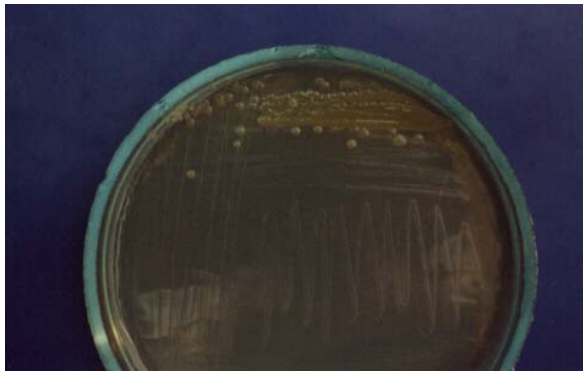


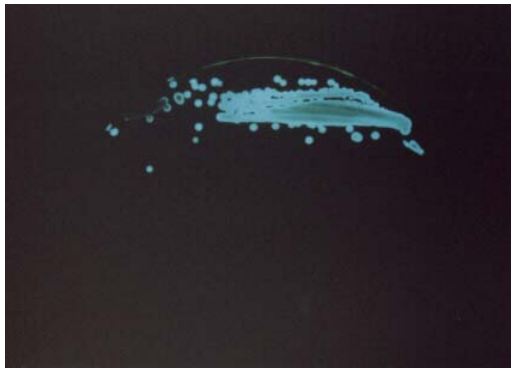


TEA 038-9



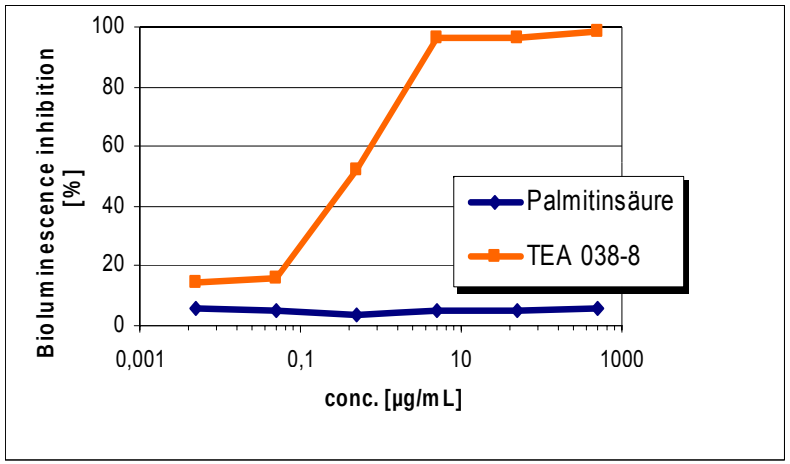
LTI074 A2

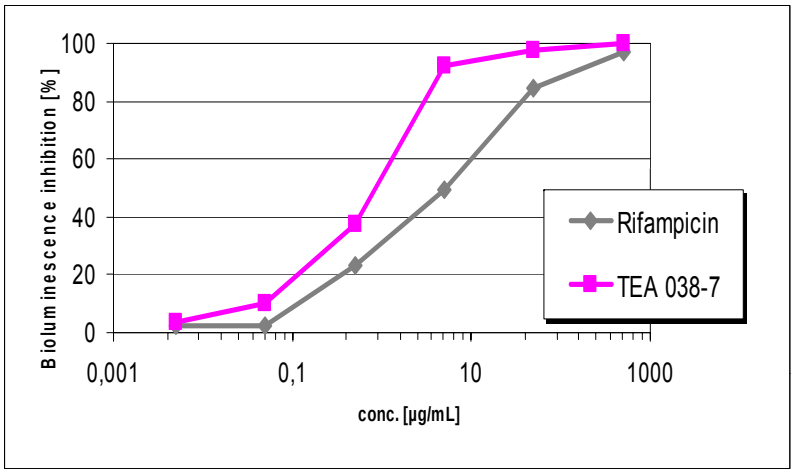


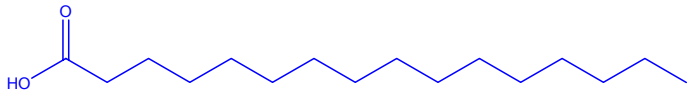


Bioactivity of the isolated fatty acids

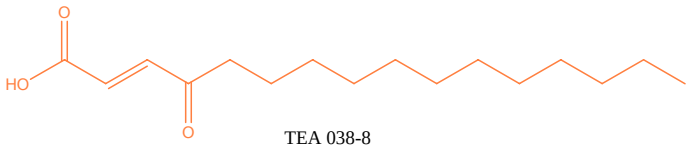
Bactericidal activity: *Vibrio fischeri* (-)*:* 24 h assay



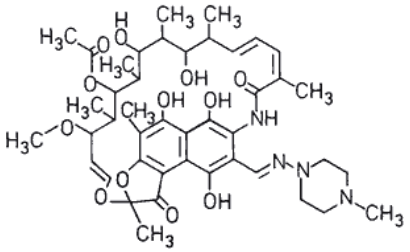




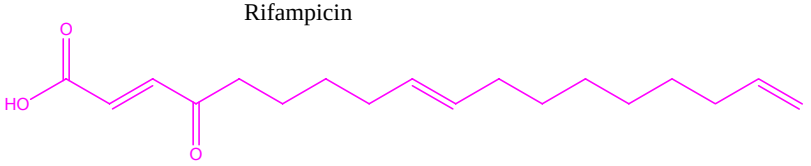
Palmitinic acid



TEA 038-8



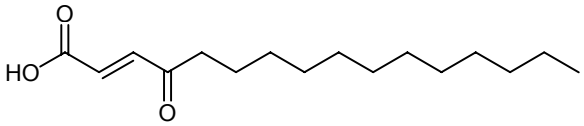
Rifampicin

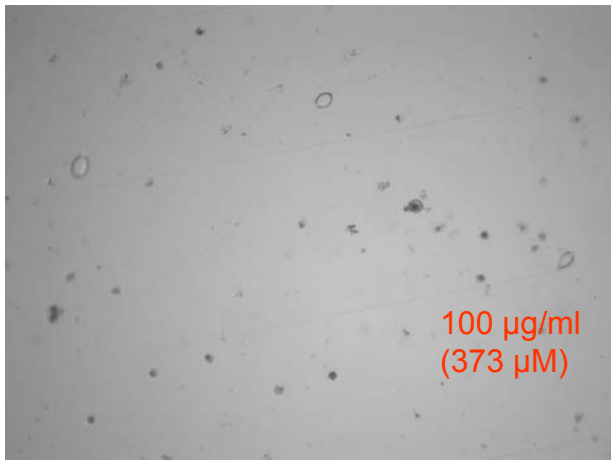


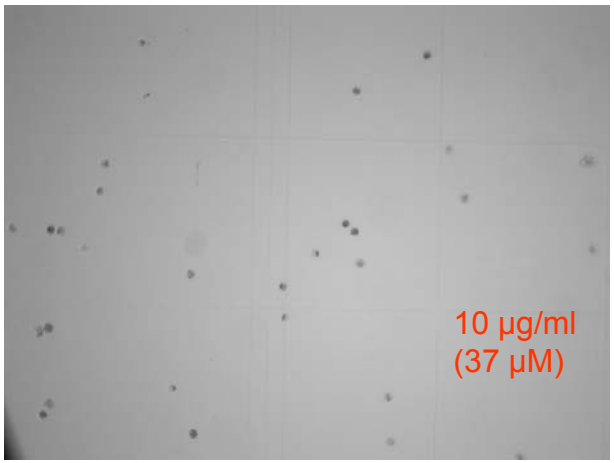
TEA 038-7

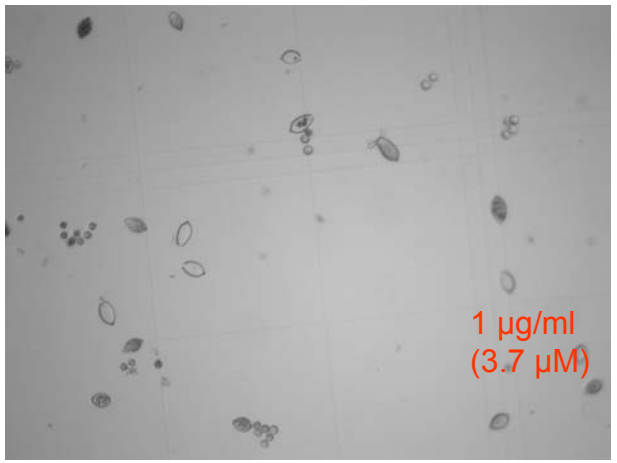
Bioactivity of the isolated fatty acids

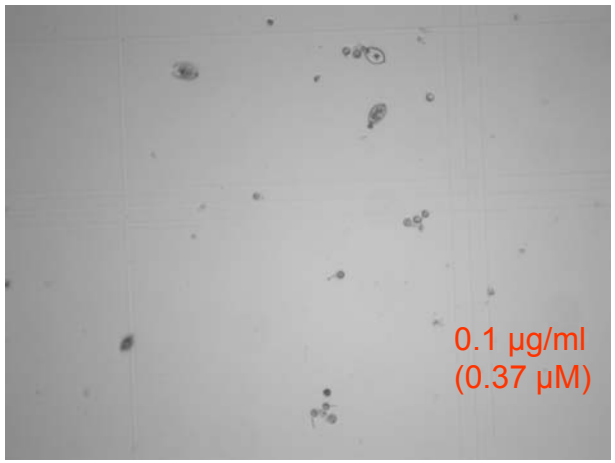
P. infestans spore germination assay

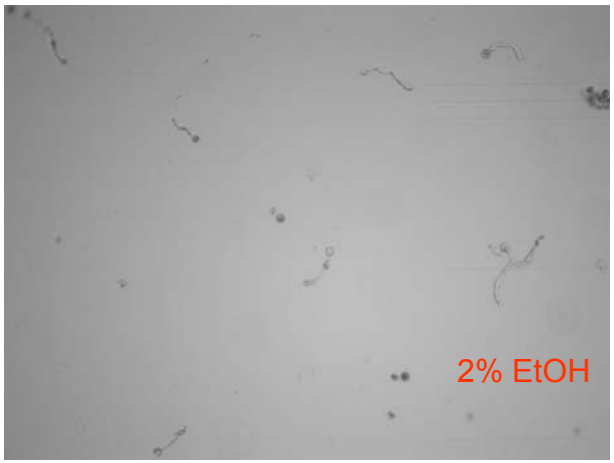


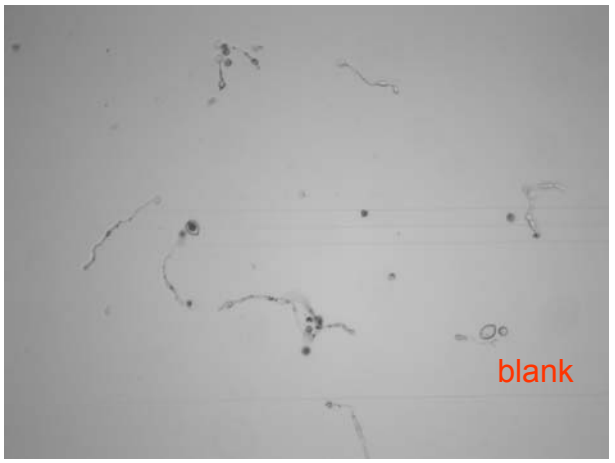






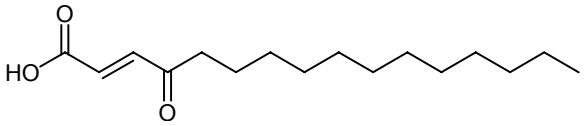


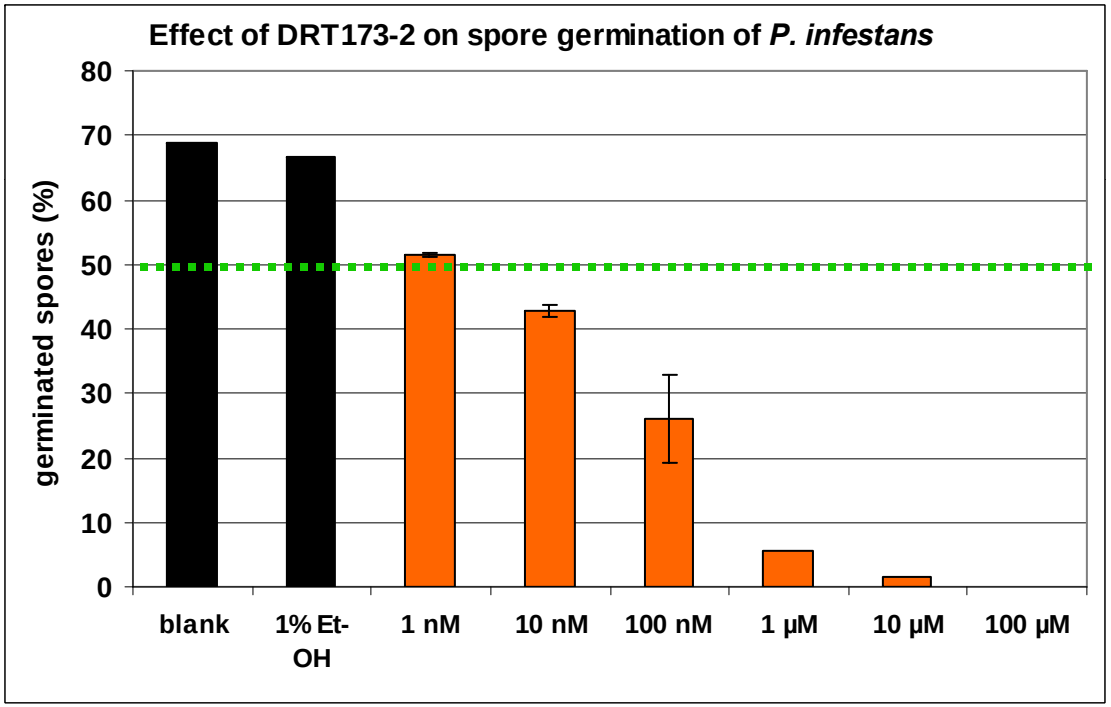




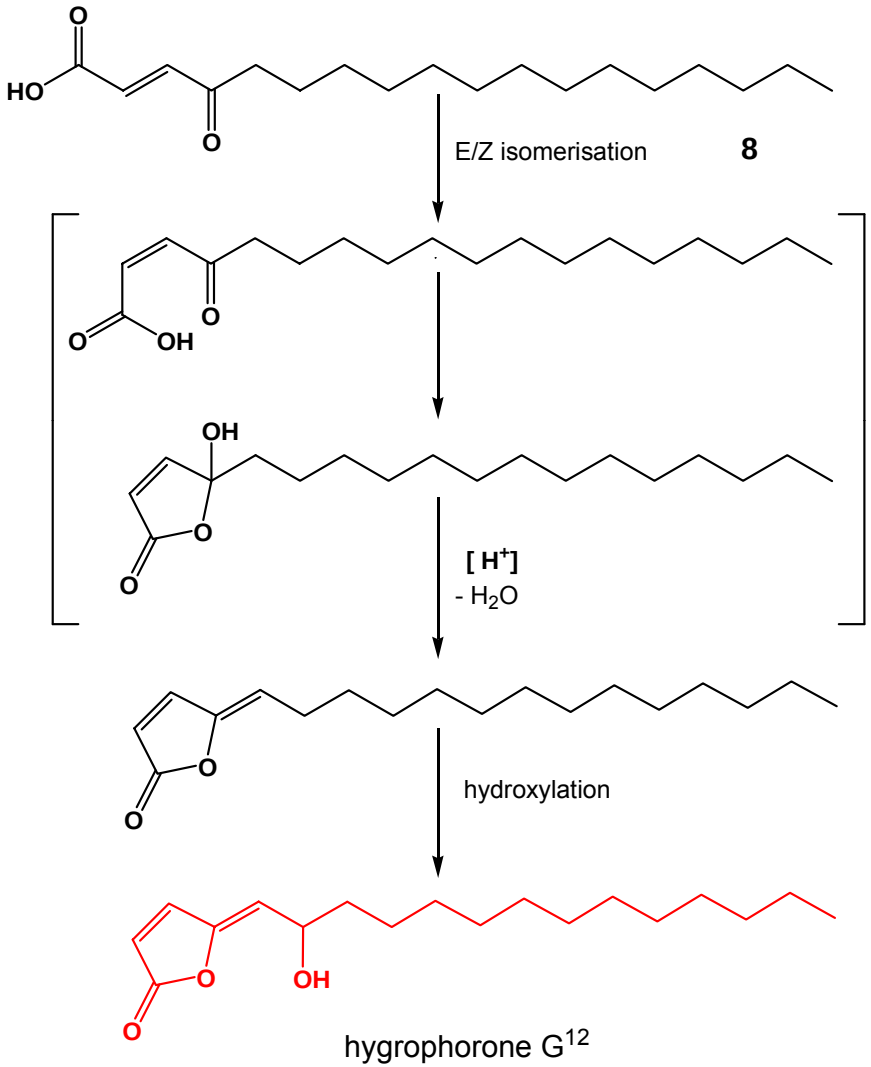
Bioactivity of the isolated fatty acids

P. infestans spore germination assay





Hypothetical relationship of (8) to hygrophorone G^{12}



hygrophorone G^{12}

Spotting of the crude extracts or compounds on TLC plates

From fungal fruit body to fungicide -

the Strobilurin-story

Strobilurus tenacellus (Pers.) Singer: „pine cone fungus“

From fungal fruit body to fungicide -

the Strobilurin-story

Strobilurus tenacellus (Pers.) Singer: „pine cone fungus“

1976: fungicidal properties of *S. tenacellus*

(Prof. T. Anke)