



International Symposium for Thin Layer
Chromatography

October 15-18th 2003, Lyon

TLC METHODS TO MONITOR MICROBIAL TRANSFORMATION PROCESSES

*Balázs Erdélyi, Antal Szabó, László Birincsik,
János Salát and Gábor Seres*

IVAX Drug Research Institute, Budapest, Hungary

e-mail: Balazs.Erdelyi@idri.hu

Analytical monitoring of two typical biotransformations

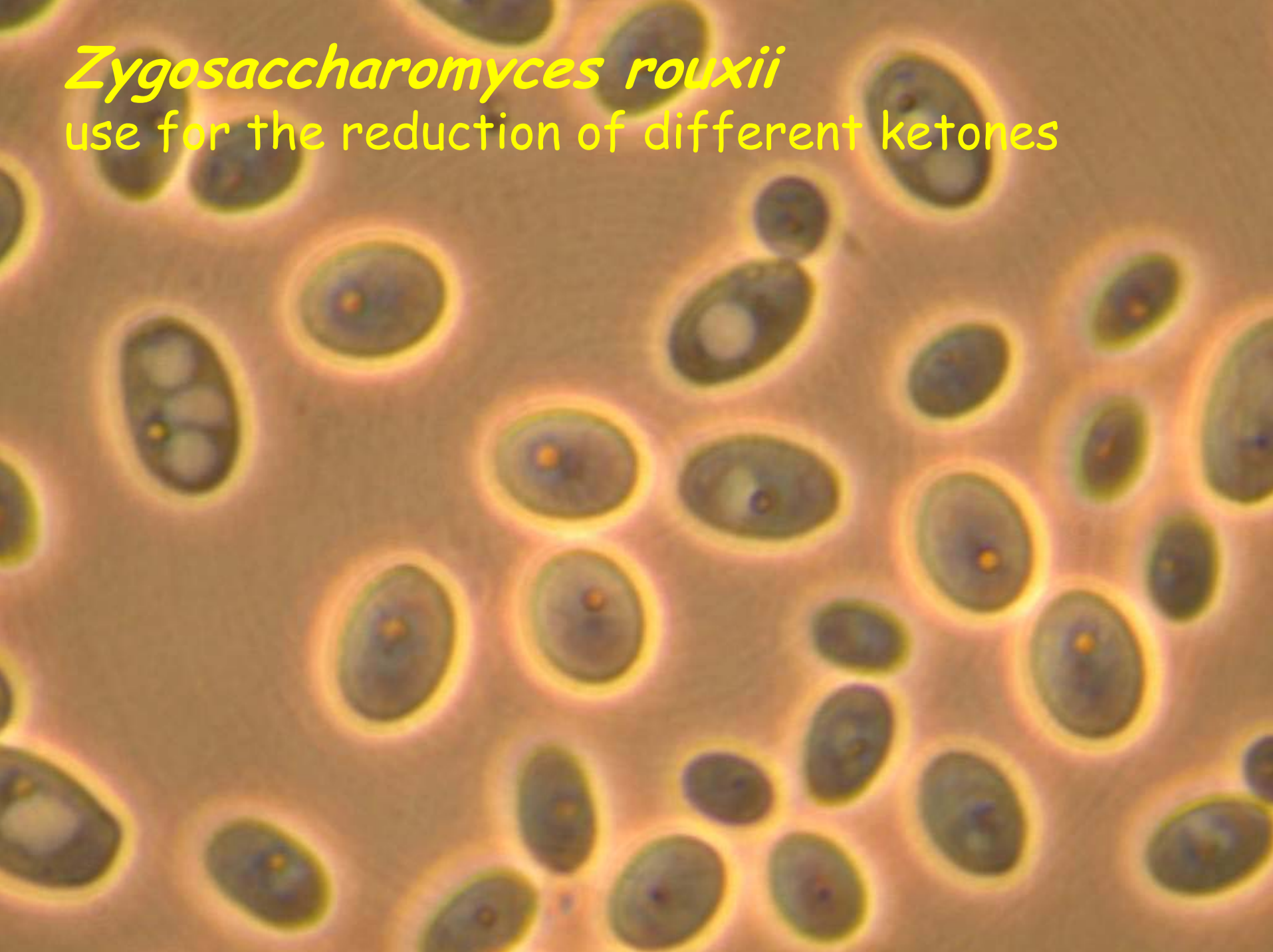
1. stereospecific **reduction** of some ketones
2. **oxidation**: regio- and stereospecific hydroxylation of compactin into pravastatin

The advantages of TLC

1. **Simplicity:** no laborious and time consuming sample preparation
2. **Productivity:** simultaneous processes with several samples, and references
3. **Flexibility:** evaluation variabilities with a single chromatogram
4. **No decrease in resolution:** using new, intact adsorbent for each run

Zygosaccharomyces rouxii

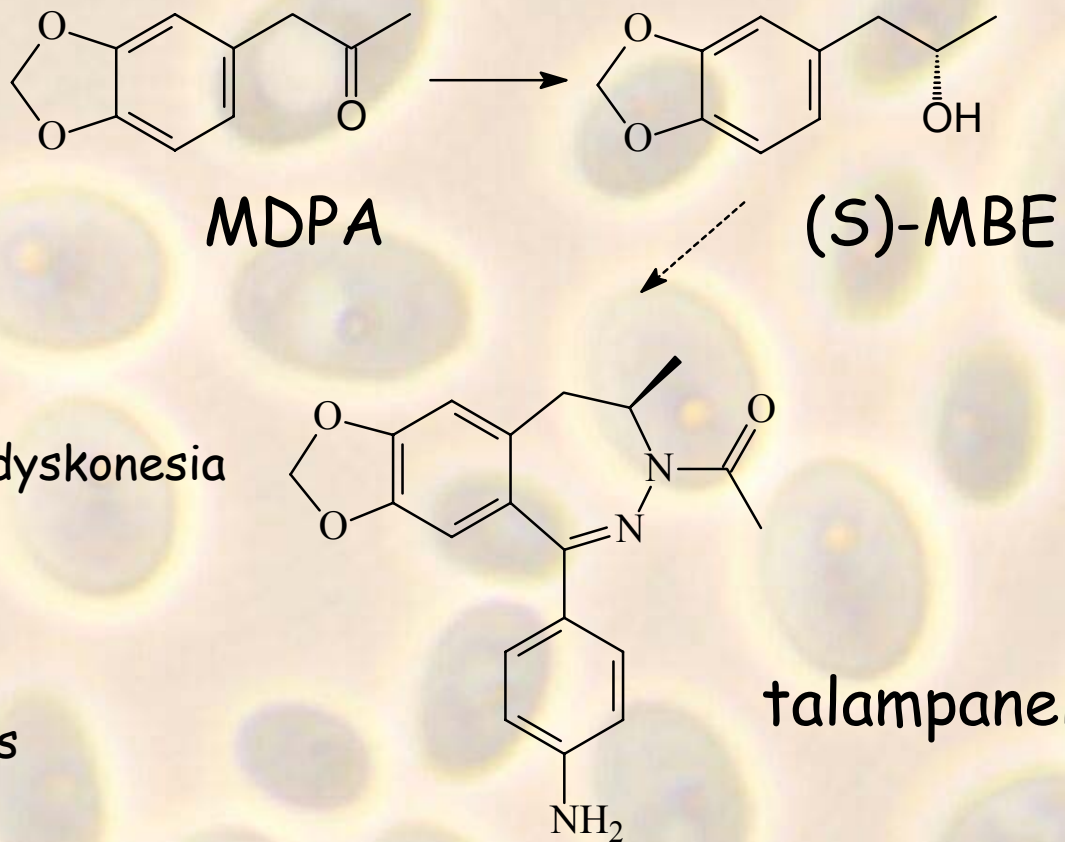
use for the reduction of different ketones



Talampanel: new drug with a wide action spectrum

Talampanel:

- AMPA receptor antagonist
- an expanded Phase II Clinical Trial for epilepsy
- Phase II Clinical Trial to treat Parkinson's Disease-associated dyskinesia
- Initiation Phase II Clinical Trial for Brain Cancer
- further investigations on Talampanel related molecules



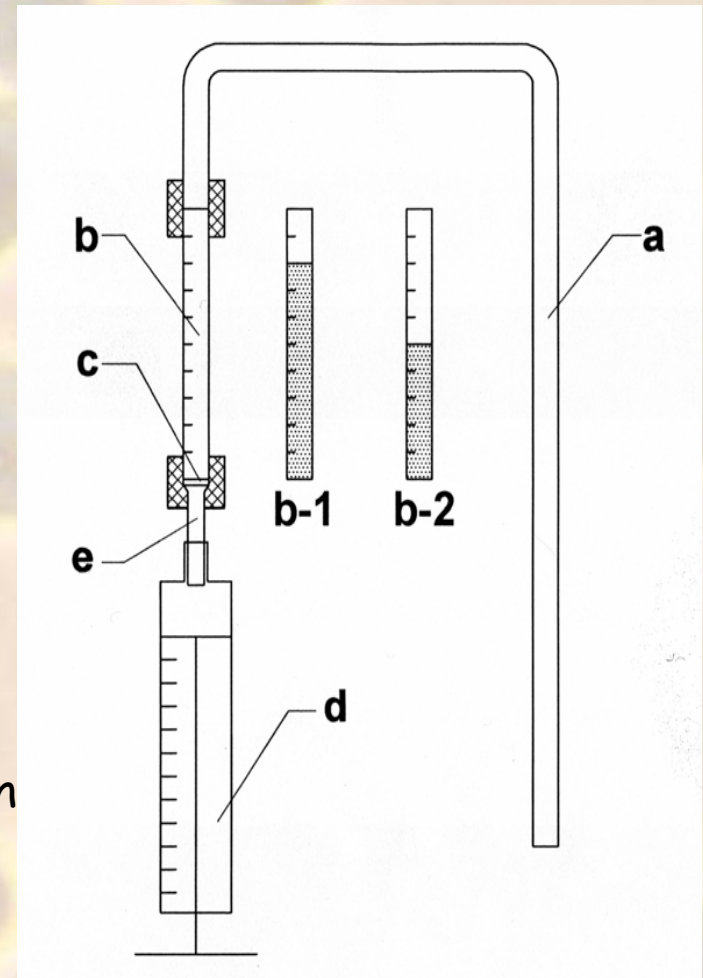
Sampling device developed for resin-based processes

This device is suitable for:

- (i) sucking
- (ii) washing
- (iii) elution
- (iv) resin vol. estimation

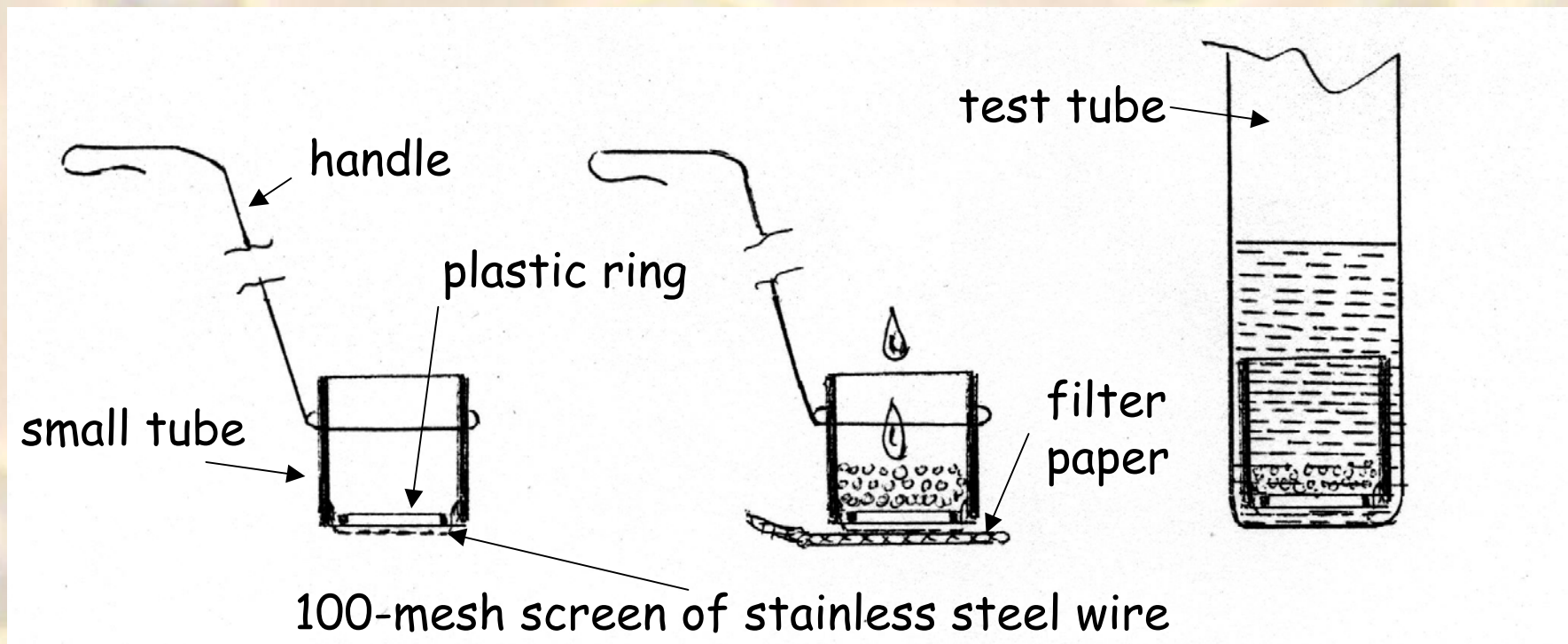
Parts of the sampler:

- (a) glass suction pipe
- (b) graduated tube
- (c) stainless steel screen
- (d) PP syringe
- (e) small PP pipe

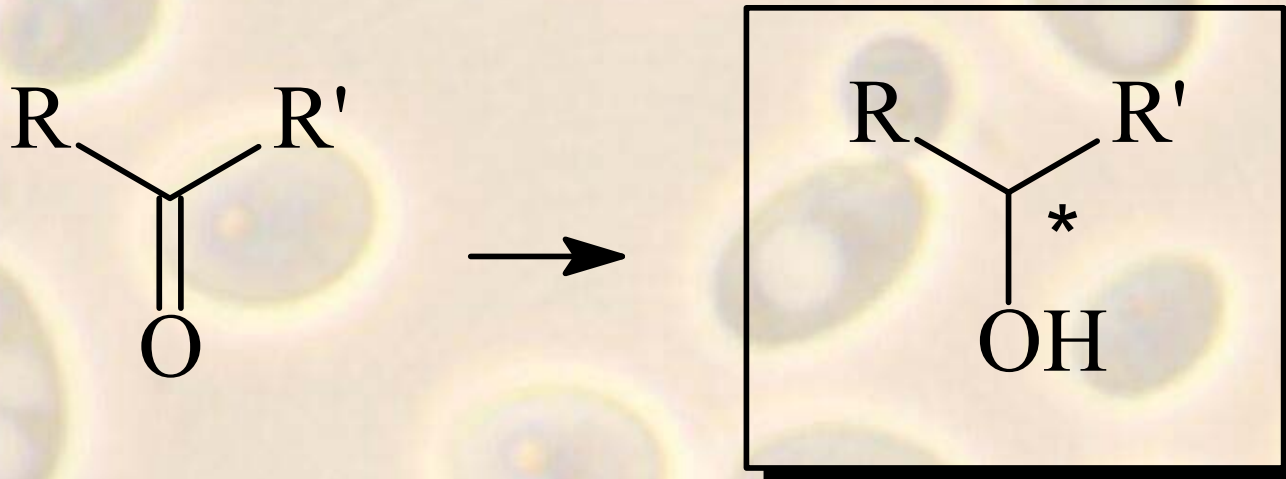


Simplified device for sampling from resin-based bioreduction media

Samples in large numbers required a further simplification of the sampler.

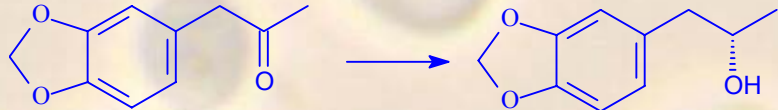





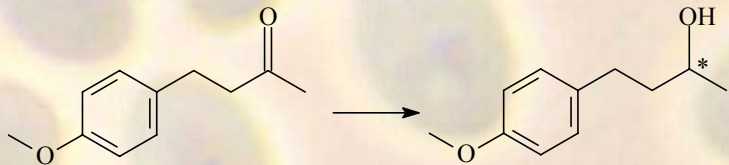


GENERAL REDUCTION SCHEME



Conditions of bioreductions:

- with *Z. rouxii* whole cells
- *in-vivo* co-factor regeneration
- with use of XAD-7 resin
- pH regulation with buffers
- flexible aeration and temperature conditions

Name of the ketones investigated	Signs of the ketones	Reduction schemes
3,4-methylenedioxy-phenylacetone	MDPA	
phenylacetone	PA	
4-methoxy-phenylacetone	MPA	
4-chloro-phenylacetone	CPA	
3,4-dimethoxy-phenylacetone	DPA	
bensylacetone	BA	
anisylacetone	AA	

TLC method to monitor the bioreductions

Spotting: CAMAG Linomat IV.

Developing on Merck F₂₅₄ plate,

Evaluation: CAMAG Scanner II.

operated with Cats 3.0 software

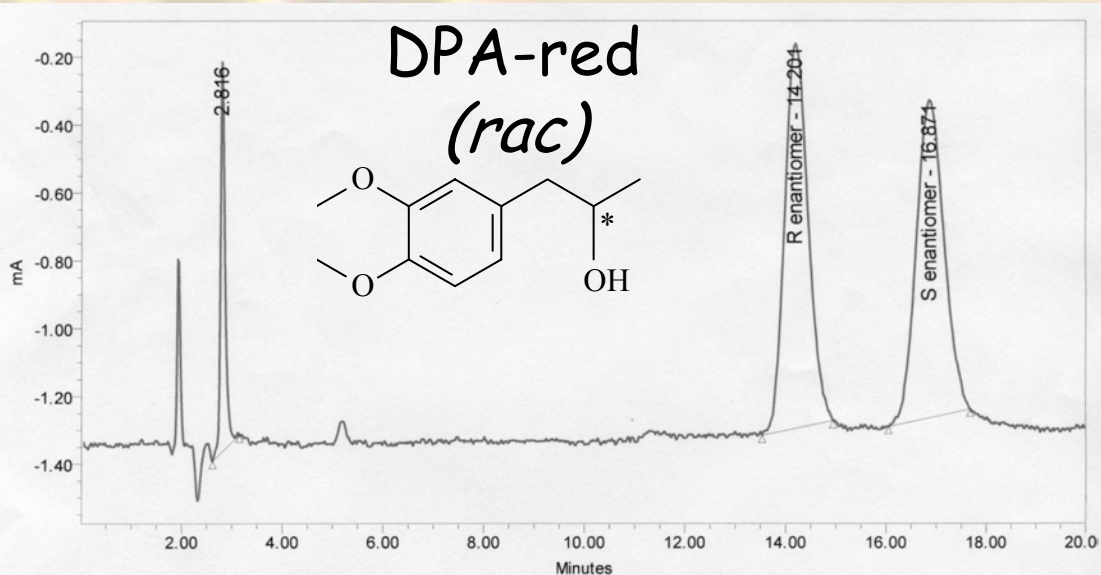
Solvent mixture:

toluene	92 v/v
tetrahydrofurane	4 v/v
methylene chloride	4 v/v

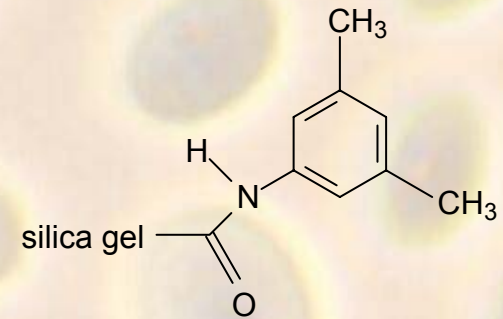
The R_f values of the ketones investigated

	Ketone	Alcohol
Signs	R_f	R_f
MDPA	0.72	0.48
PA	0.79	0.50
MPA	0.92	0.60
DPA	0.50	0.33
CPA	0.78	0.48
AA	0.73	0.41
BA	0.80	0.46

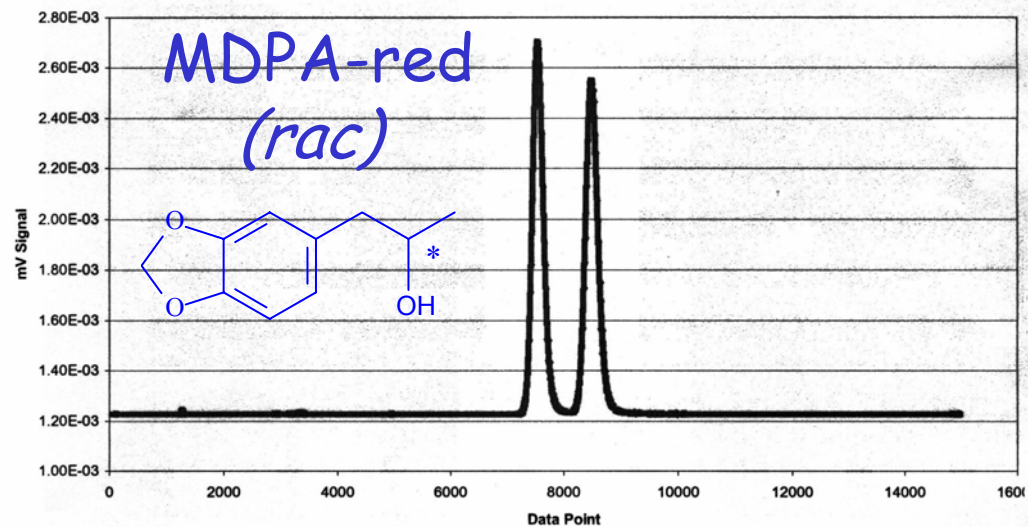
Stereospecific analysis



Column: DAICEL, Chiralcel OD-H
cellulose tris (3,5-dimethylphenylcarbamate)

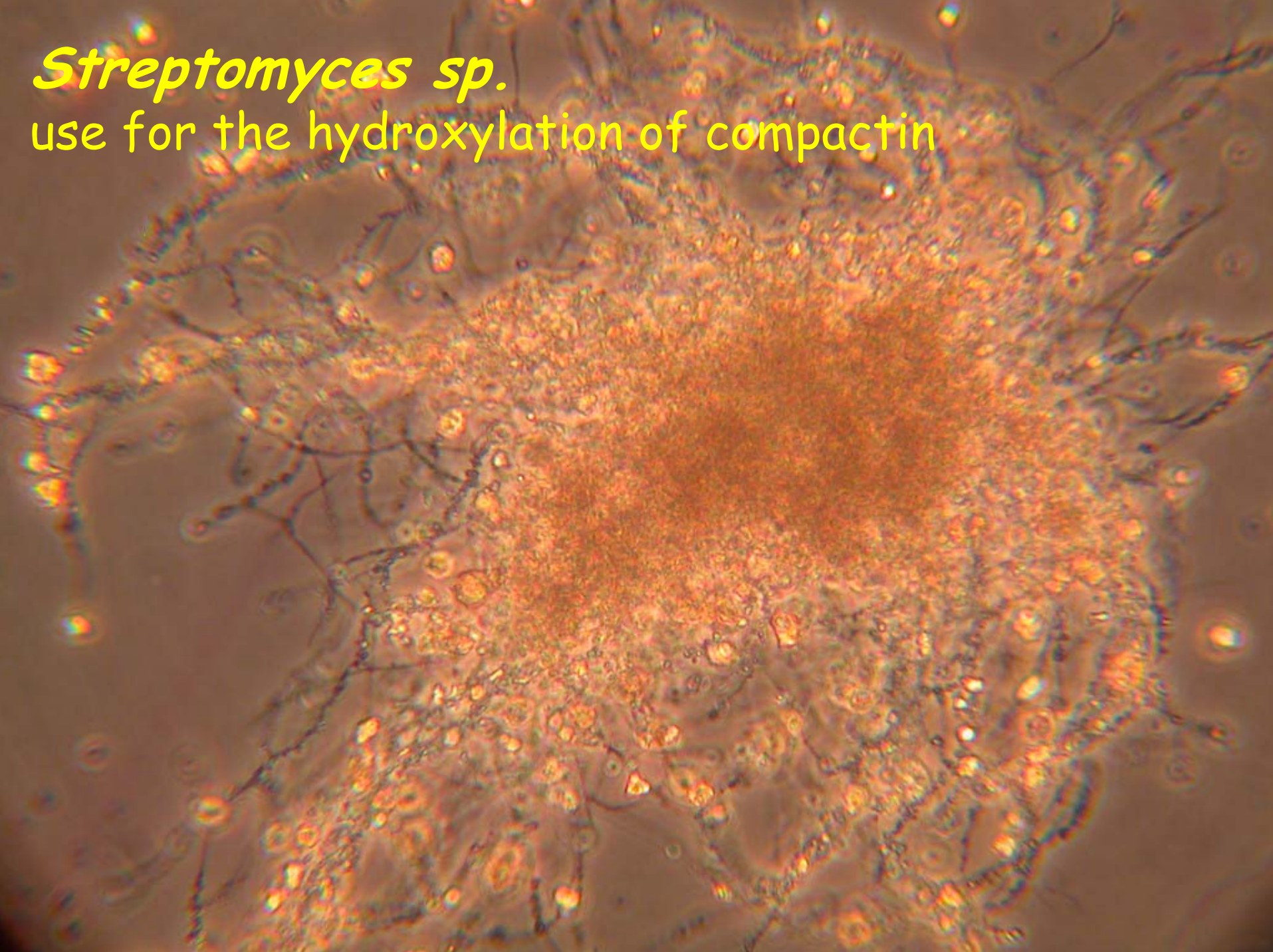


mobile phase: hexane 98 v/v
i-propanole 2 v/v

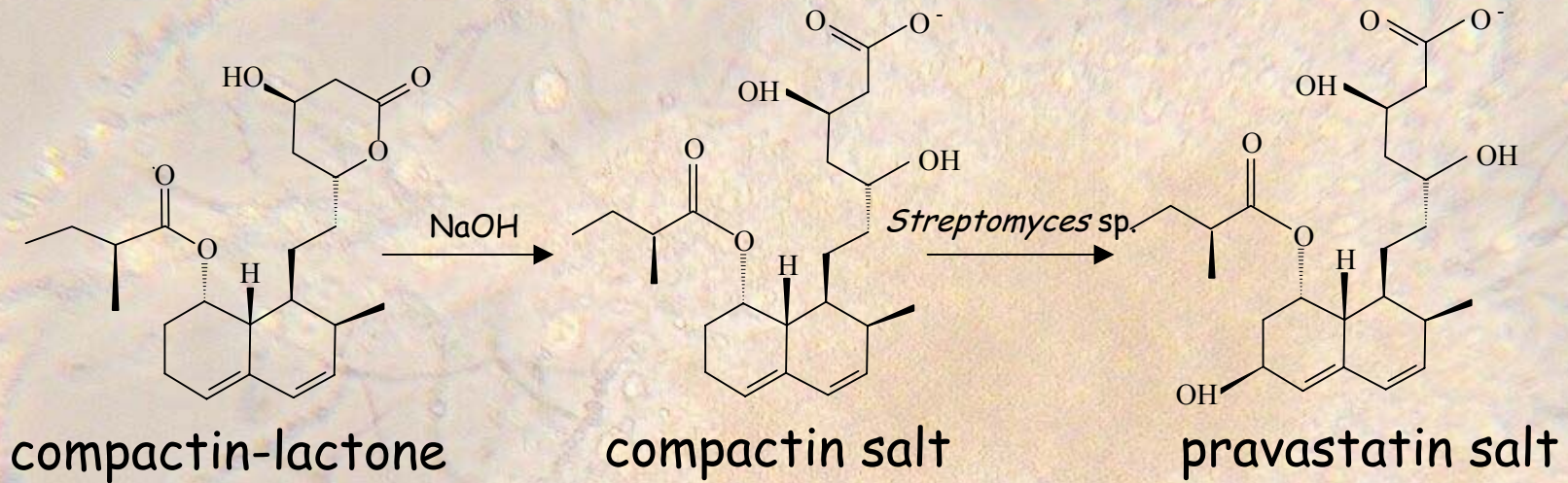


Streptomyces sp.

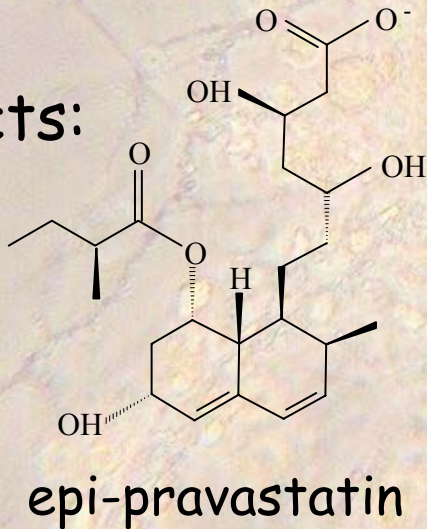
use for the hydroxylation of compactin



Reaction scheme of pravastatin production and two by-products often formed in the hydroxylation process



By-products:

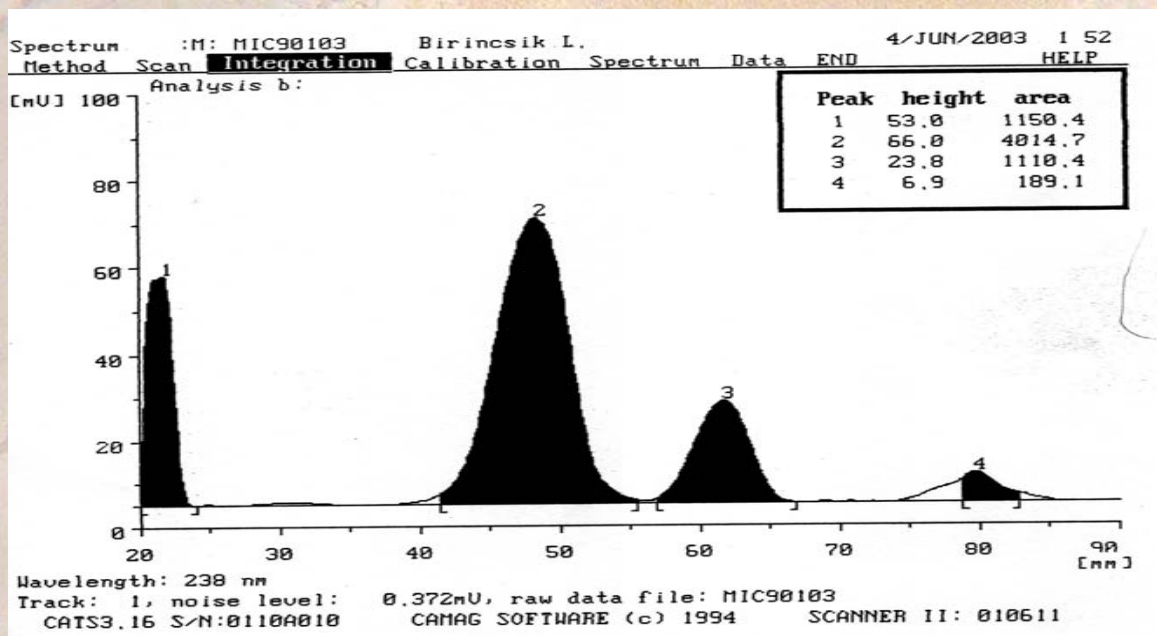


Rapid method for quantitative monitoring of compactin hydroxylation

Solvent mixture

toluene 30 v/v
acetone 20 v/v
glacial acetic acid 1 v/v

Merck Silicagel plate No. 5729
migration distance: 70 mm
densitometry at 238 nm

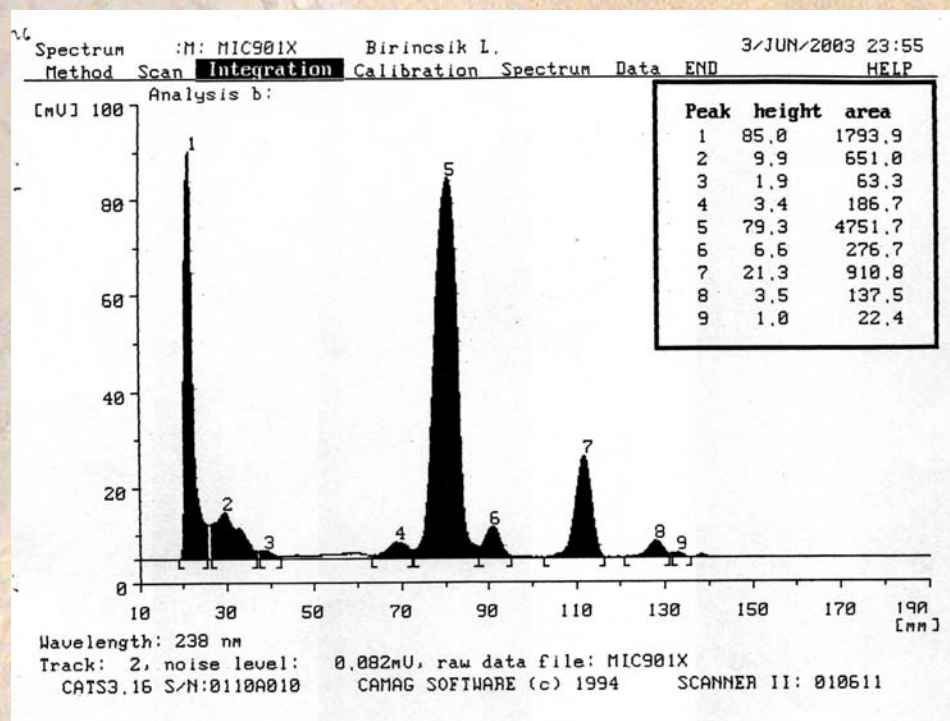


HPTLC method for monitoring of the by-products and impurities

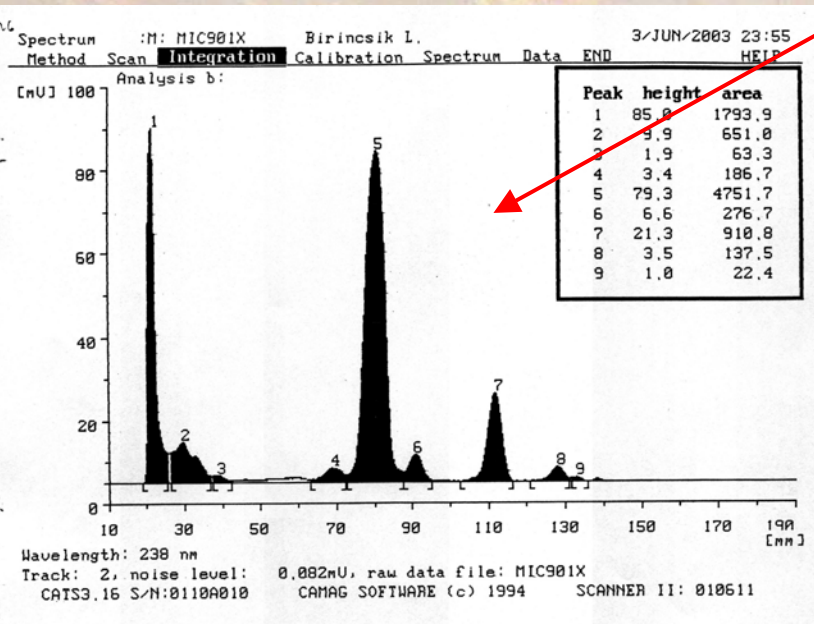
Solvent mixture

Toluene 30 v/v
Acetone 20 v/v
glacial acetic acid 1 v/v

Merck Silicagel plate **No. 5626**
migration distance: **180 mm**
densitometry at 238 nm

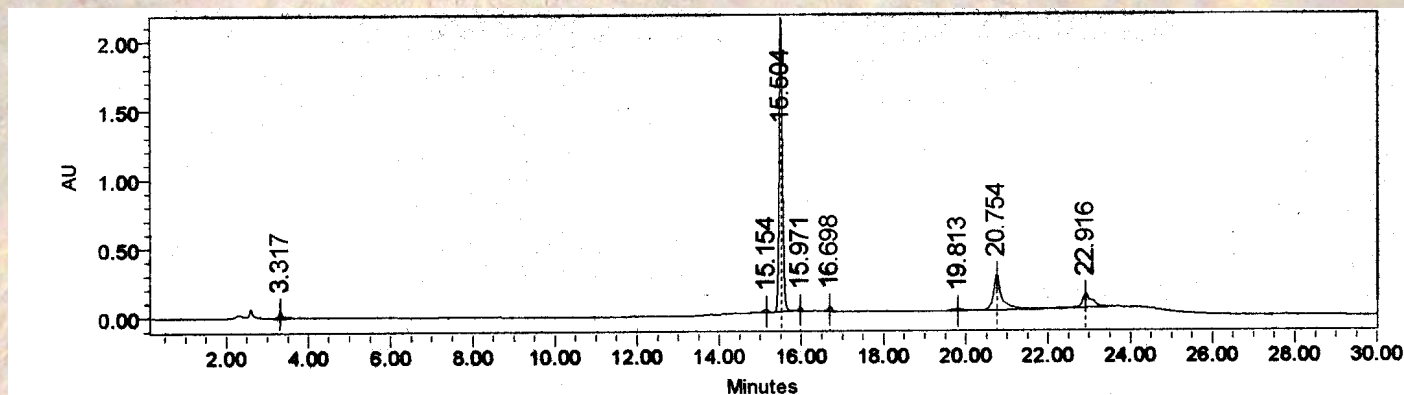


Resolution of HPTLC and HPLC



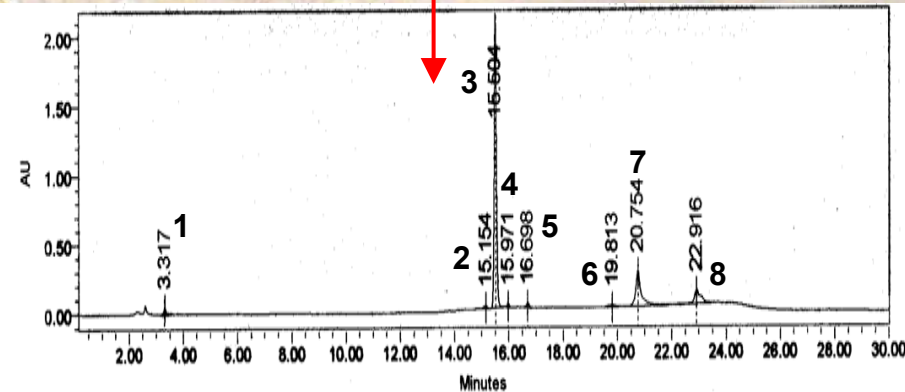
Peak No.	Compound
7	compactin
5	pravastatin
6	epi-pravastatin
1-3	impurities from ferm. broth
4,8,9	by-products

Nine peaks were separated from the fermentation broth by HPTLC technique.

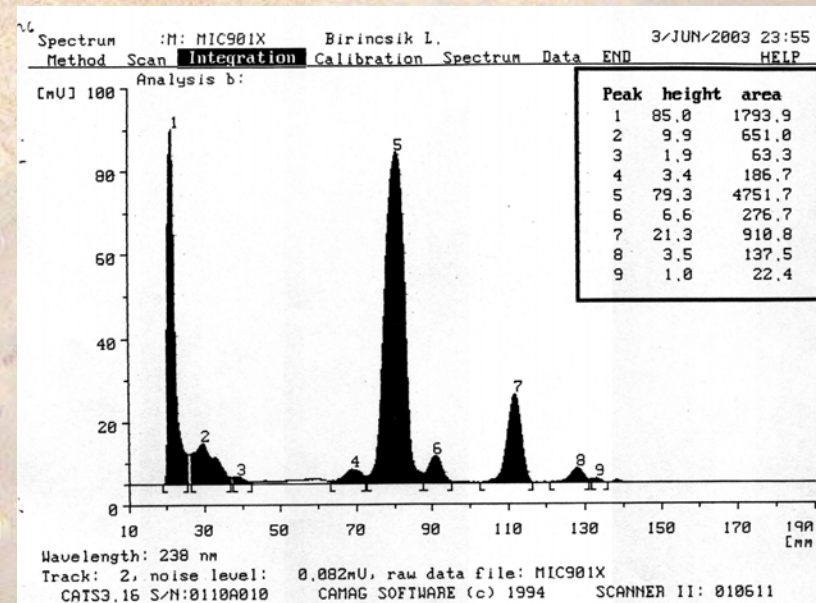


Resolution of HPTLC and HPLC

Peak No.	Compound
7	compactin
3	pravastatin
4	epi-pravastatin
1,8	impurities from ferm. broth
2,5,6	by-products



Eight peaks were separated by HPLC technique applying new column.



epi-pravastatin:

TLC: present

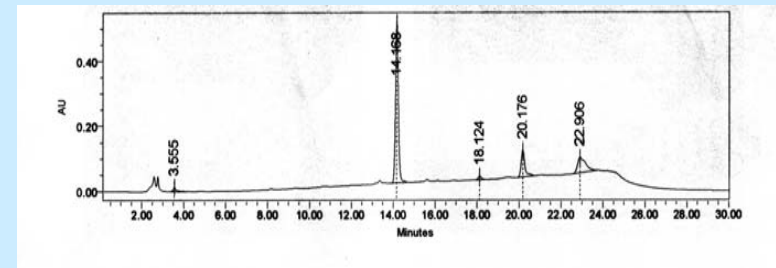
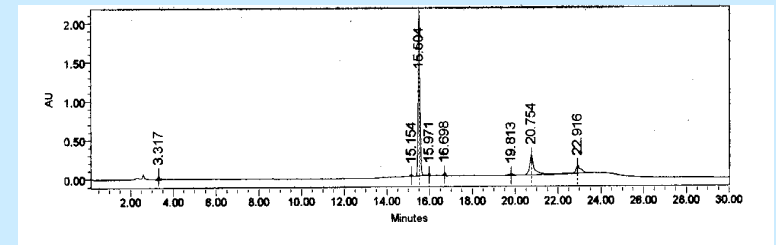
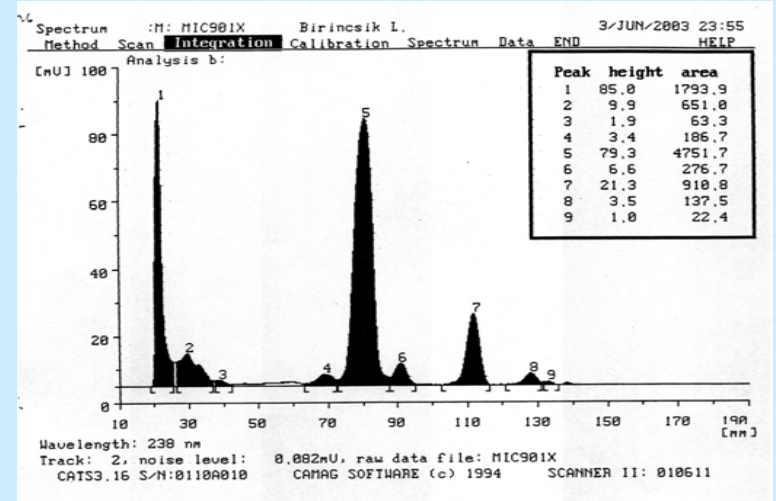
HPLC (new): present

HPLC (used): absent

„polar group“:

TLC: high intensities

HPLC: low intensities



Summary: (HP)TLC or HPLC in a microbial development?

- TLC methods are suggested for monitoring biotransformation processes
- HPLC (or GC) for the chiral analysis from the purified products