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On the mechanism of inter-kingdom signalling - synthesis of isotope labelled *N*-acyl-L-homoserine lactones (AHLs)

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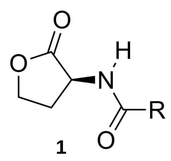
Introduction

***N*-acyl-L-homoserine lactones 1 (AHLs)** are natural products which



R – acyl or alkyl chain

belong to **semiochemicals** (signal molecules or infochemical compounds). They act as messengers within (**pheromones**) or between (**allomones**) species.

AHLs are so-called autoinducer molecules, enabling inter-bacterial communication (**Quorum Sensing**) and inter-kingdom communication (**Inter-kingdom Signalling**).

Inter-bacterial communication - biofilm formation

Biofilm - an aggregate of microorganisms (Fig. 1-3)

- Common cause of persistent infections
- Chronic, destructive inflammatory processes
- Antibiotic resistant

Fig. 1. Biofilm

Fig. 2. Biofilm

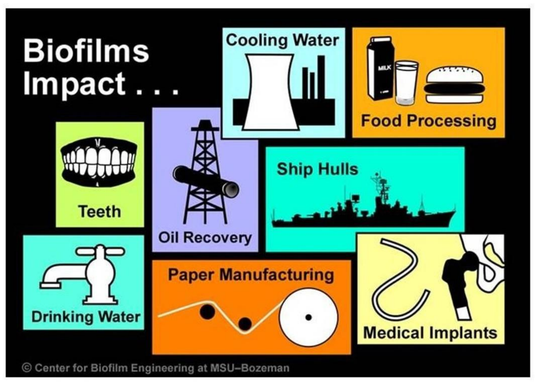


Fig. 3. Occurrence of biofilm

Inter-kingdom Signalling – communication between Prokaryotes and Eukaryotes

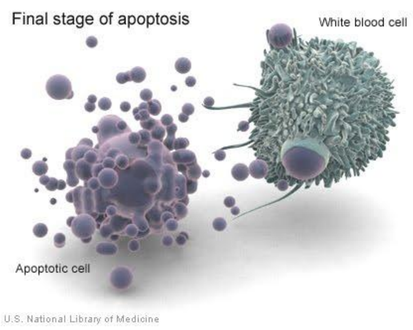


Fig. 4. Apoptosis of the cell

Interactions of bacteria with a variety of mammalian cells:

- Induction of apoptosis (Fig. 4)
- Induction of the chemotaxis of neutrophils (Fig. 5,6)

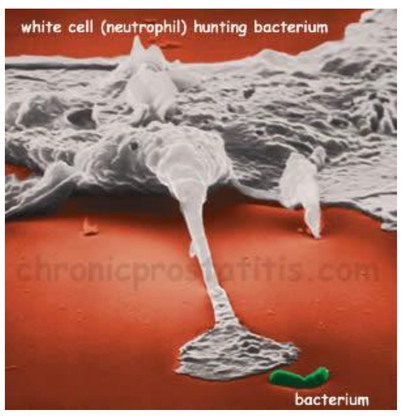


Fig. 5. Chemotaxis of neutrophils

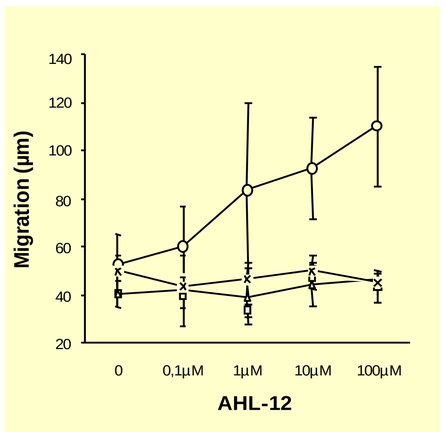


Fig. 6. Migration of human polymorphonuclear neutrophils (PMN) toward increasing concentrations of AHLs with vary chain length.

Aim

Synthesis and isotopic labelling of *N*-acyl-L-homoserine lactones - detection of AHLs crossing eukaryotic cell membranes

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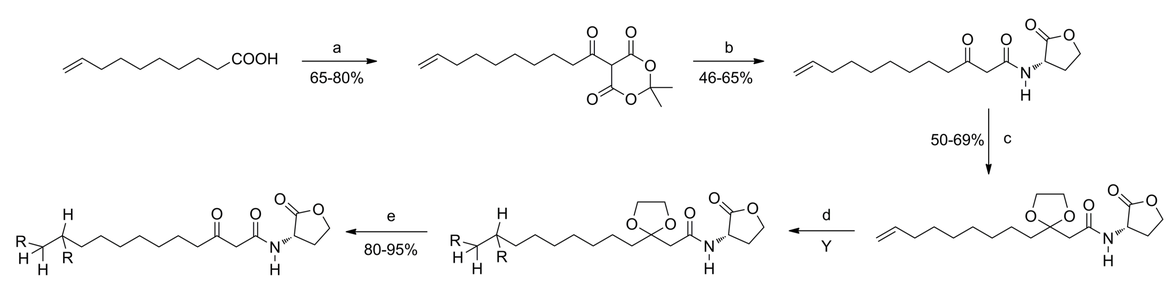
Elucidating the mechanism of Inter-kingdom Signalling

Results and discussion

Synthesis of a highly biologically active, deuterium and tritium labelled *N*-(3-oxododecanoyl)-L-homoserine lactone (scheme 1).

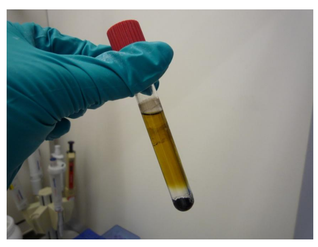


Scheme 1. a) 4-Dimethylaminopyridine (DMAP); 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC); CH_2Cl_2;RT; b) L-homoserine lactone hydrobromide, Et_3N; CH_3CN; RT-80˚ C; c) Ethylene glycol, *p*-TsOH, $CH(OMe)_3$, PhMe, 110˚ C-RT; d) $Pd(OAc)_2$, THF, CH_3COOH, MeOH, $NaBR_4$, $NaOH_{aq}$, -196 ˚ C – RT; e) $HClO_4$, CH_2Cl_2, 0˚ C-RT; R = D or T; Y = yield (see Table 1).

Unconventional conditions of the reaction (Fot. 1, 2, 3).

Fot. 1. Reaction starts in the liquid nitrogen.

Fot. 2. Agitation in a room temperature.

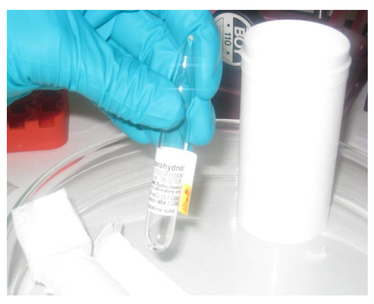


Fot. 3. Reduced palladium residue.

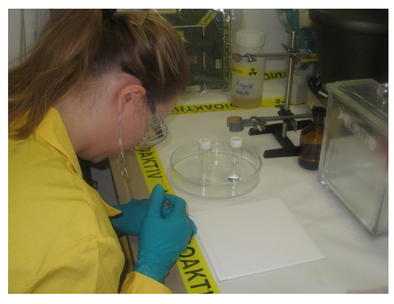
Step d									
Entry	**R**	**Time [h]**	**Additives notes**	**Other deuterated reagents**	**Yield [%]**	**D content[a] at preterminal C atom [%]**	**D content[a] at terminal C atom [%]**	**Isomers[b]**	**Specific radioactivity[c] [mCi/mmol]**
1	D	16	$NaBD_4$	-	65	45-60	99	**[M+(1-6)+H+] [M-(1,2)+H+]**	-
2	**D**	**16,5**	**Excess of $NaBD_4$**	**1M NaOD (30% NaOD in D_2O diluted in MeOH)**	**79**	**90**	**99**	**[M+(1,2)+H+] [M-(1,2)+H+]**	**-**
3	**T**	**16**	**$NaBT_4$**	**-**	**78**	**-**	**-**	**[M+1+H+] [M-1+H+]**	**588,5**

Table 1. Results for the synthesis of the deuterium and tritium labelled *N*-(3-oxododecanoyl)-L-homoserine lactones ; [a] Determined by ^{1}HNMR and mass spectrometry. [b] Determined by ESI-TOF MS. [c] Determined by Liquid Scintillation counter.

Work with tritium - the radioactive isotope (Fot. 4, 5, 6).



Fot. 4. Sodium borotritide ($NaBT_4$).



Fot. 5. Preparative thin layer chromatography.

Fot. 6.Scintillation counter - measurement of radioactvity.

Conclusions

- The new methods of isotopic labelling of AHL was developed. The methods are efficient and enable further biological investigations;
- Structures of the products were confirmed by TLC, ^{1}H NMR, ^{13}C NMR, ESI-TOF MS, HRMS, IR and Raman spectroscopy, TLC / autoradiography and Liquid Scintillation Counter.

References

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