Towards the synthesis of mannose derivatives of natural phenolic compounds

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Objectives

The aim of this project is to graft a sugar moiety onto polyfunctional natural phenolic compounds (see examples in figure 1). This should enhance their water solubility and the choice of an adequate sugar such as mannose could provide cellular recognition. The synthesis route was first tested on cinnamyl alcohol which is structurally close to the base pattern of the molecules in figure 1.

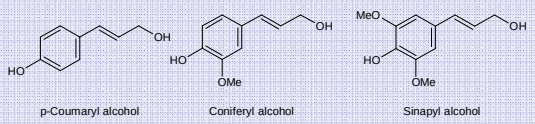
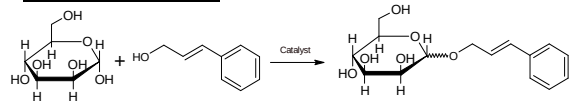


Figure 1: Structures of natural phenolic compounds

Synthesis of cinnamyl mannoside

Reactionnal Scheme



Experimental

	cinnamyl alcohol	D-mannose	catalyst	water	solvent	During, temperature
Enzymatic -catalyzed synthesis conditions (Akita's method, 2006)	0.8M	0.2M	β-D-glucosidase from almond (12U/ml)	10%	t-butanol	7 days, 50°C
Chemical synthesis conditions (Richel's method, 2010)	0.8M	0.2M	Immobilized acid catalyst (35%, 8mg/ml)	none	t-butanol	7 days, 50°C

Both synthesis were also performed in MicroWave assisted (MWA) conditions (45 min, 50°C, 200W)

Analysis conditions:
- HPLC Agilent 1200 Series, Agilent Eclipse XDB C18 column (5µm, 4.6*150mm), gradient elution with water and acetonitrile (start 5% -> end 100%) at 0.6ml/min
- ESI-MS data acquired with a Bruker spectrometer, N2 (30°C) as nebulization and drying gaz,scan range between 100 and 1000 m/z

Comparison of the chemical and enzymatic synthesis routes

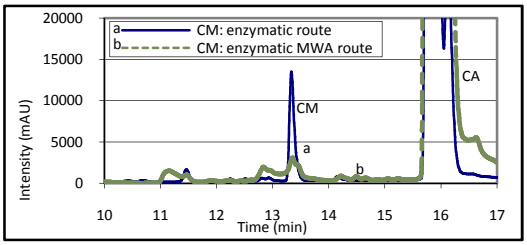


Figure 2: CM synthetized by the enzymatic route

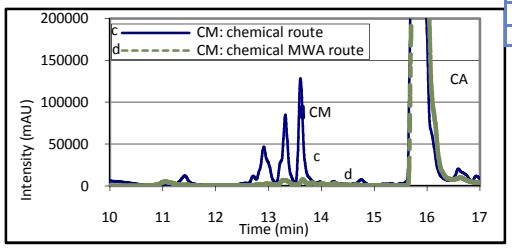
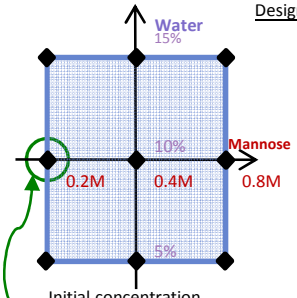


Figure 3: CM synthetized by the chemical route

MS data negative mode	
Detected m/z	Attribution
294.9	$[CM-H]^-$
341.0	$[CM+HCOO]^-$

- The enzymatic route seems to be more specific as only one product peak (CM) is observed (at least 5 by the chemical route)
- MWA synthesis do not improve CM production in tested conditions.

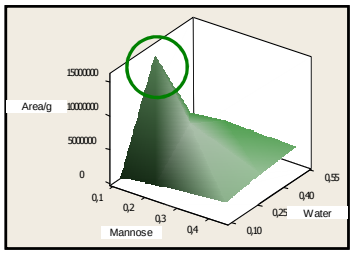
Optimisation of mannose and water levels for the enzymatic route



Initial concentration conditions (Akita, 2006) => best CM production

Design of experiment

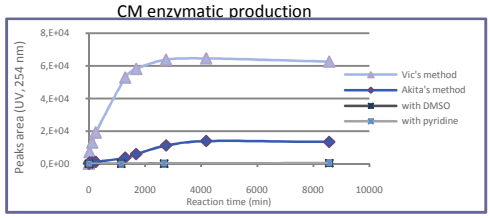
Optimum obtained with 0.2M mannose and 10% water



Comparison of two methods for the enzymatic synthesis

β-D-glucosidase from almond (12U/ml) + D-mannose (0.2M) + water (10%)
[+CA (0.8M) + t-BuOH (Akita's method) [+co-solvent (pyridine or DMSO)]
+CA (as solvent and reagent) (Vic's method)
Reaction for 7 days at 50°C

CM enzymatic production



- Best CM production obtained when using the CA as solvent
- However, tert-butanol is necessary to solubilize solid alcohol reagents
- No activity was observed in the presence of Pyridine or DMSO.

Conclusions

- Results show that β-glucosidase is able to synthetize CM from M and CA
- Enzyme-catalyzed route lead to only one product and is so more specific than the chemical route where several products are observed.

Perspectives

- This reaction will be tested with more complex molecules (for example coniferyl alcohol)
- Obtention of only one product will be important for next fundamental studies (Interaction of the product with model membranes by Isothermal Titration Calorimetry and with the Langmuir Film Trough technique)

References

Akita, H. *et al. Journal of Molecular Catalysis B: Enzymatic* **2006,** *40*, 8–15
Richel, A. *et al. Tetrahedron Letters* **2010,** *51*,10, 1356-1360
Vic, G.; *et al., Carbohydrate research* **1995,** *279*, 315-319.

Acknowledgments

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