Text on vertical bar

Biotransformations from and to methylated flavonoids

Hpw all went to shit

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noch nicht bekannt

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Preface

1 Abstracts

1.1 English Abstract

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1.2 Deutsche Zusammenfassung

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II

Thesis

2 Introduction

Some introductionary text

2.1 Natural products and secondary metabolites

2.1.1 General

2.1.2 Classes of natural products

Terpenoids and Steroids

... here is some text

Polyketides and non-ribosomal peptides

... here is some text

Alkaloids

... here is some text

Phenylpropanoids

... here is some text

2.2 Alkylating reactions in nature

2.2.1 Methylation

2.2.2 Prenylation

2.2.3 Glycosylation

2.3 Usage and expansion of natures reaction toolbox

2.3.1 Terpene synthases and elongases

2.3.2 Methyl transferases

2.3.3 Glycosyl transferases

2.3.4 Other important enzymes in biotech research

BMVOs

Esterases/Lipases

Oxidases

Lyases

Transaminases

2.4 Conclusion

3 Material And Methods

3.1 Materials

3.1.1 Chemicals

Enzymes and buffers used for molecular cloning were obtained from Thermo Scientific (Darmstadt, Germany), unless otherwise noted. Flavonoid HPLC standards were purchased from Extrasynthese (Genay, France). Deuterated solvents were aquired from Deutero GmbH (Kastellaun, Germany). Solvents, purchased from VWR (Poole, England), were distilled in-house before use.
All other chemicals were obtained from either Sigma-Aldrich (Steinheim, Germany), Applichem (Darmstadt, Germany), Carl Roth (Karlsruhe, Germany) or Merck (Darmstadt, Germany).

3.1.2 Instruments

circulary dichroism (CD)-spectrometer	Jasco J-815 (Eaton, USA)
electrophoresis (horizontal)	Biometra Compact XS/S (Göttingen, Germany)
electrophoresis (vertical)	Biometra Compact M (Göttingen, Germany) Biometra Minigel-Twin (Göttingen, Germany)
fast protein liquid chromatography (FPLC)	ÄKTA purifier (GE Healthcare, Freiburg, Germany)
gas chromatography coupled mass-spectrometry (GC/MS)	GC-MS-QP2010 Ultra (Shimadzu, Duisburg, Germany)
high-performance liquid chromatography (HPLC)	VWR ???
Isothermal Titration Calorimetry (ITC)	MicroCal iTC200 (Malvern, Worcestershire, UK)
micro-titer plate (MTP)	???
MTP-reader	SpectraMax M5 (Molecular Devices, Biberach, Germany)
nuclear magnetic resonance (NMR)-spectrometer	Varian Unity 400 (Agilent, Böblingen, Germany) Varian VNMRS 600 (Agilent, Böblingen, Germany)
photospectrometer	Eppendorf Biophotometer Plus (Hamburg, Germany) JASCO V-560 (Eaton, USA) Nanodrop ???
centrifuges	Eppendorf 5424 (Hamburg, Germany) Hettich Mikro 120 (Kirchlengern, Germany) Beckman Avanti J-E (Krefeld, Germany)
centrifuge rotors	Beckman JA-10, JA-16.250, JS-4.3 (Krefeld, Germany)

3.1.3 Proteins

3.1.4 Bacterial strains

E.coli

DH5α	F$^-$ Φ80*lacZ*ΔM15 Δ(*lacZYA-argF*) U169 *recA*1 *endA*1 *hsdR*17 (rK$^-$,mK$^+$) *phoA supE*44 λ^- *thi*-1 *gyrA*96 *relA*1 Invitrogen, Karlsruhe
One Shot TOP10	F$^-$ Φ80*lacZ*ΔM15 Δ(*mrr-hsdRMS-mcrBC*) *recA*1 *endA*1 *mcrA* Δ*lacX*74 *araD*139 Δ(*ara-leu*)7697 *galU galK rpsL* (Str^R) λ^- *nupG* Invitrogen, Karlsruhe
BL21(DE3)	F$^-$ *ompT hsdSB*(r_B^-,m_B^-) *gal dcm* λ(DE3) Invitrogen, Karslruhe
JM110	*rpsL thr leu thi lacY galK galT ara tonA tsx dam dcm glnV*44 Δ(*lac-proAB*) e14- [F' *traD*36 *proAB*$^+$ *lacI*^{q} *lacZ*ΔM15] *hsdR*17($r_K^-m_K^+$) Martin-Luther-University Halle-Wittenberg
JW1593 (BW25113 derivative)	*rrnB* Δ*lacZ*4787 *HsdR*514 Δ(*araBAD*)568 *rph*-1 Δ*ydgG* (Kan^R) Keio Collection, National Institute of Genetics (Japan)
MG1655	F$^-$ λ^- *ilvG*$^-$ *rfb*-50 *rph*-1 DSMZ, Hamburg
Origami(DE3)	genotype Novagen, Wisconsin (USA)
Rosetta	genotype Novagen, Wisconsin (USA)
Rosetta pLys	genotype Novagen, Wisconsin (USA)
C41(DE3)	genotype Lucigen, Wisconsin (USA)
C43(DE3)	genotype Lucigen, Wisconsin (USA)
T7 Express	genotype NEB, Massachusetts (USA)

Agrobacterium tumefaciens

GV3101	genotype Sylvestre Marillonet, IPB

3.1.5 Plasmids

Table 3.1.: Plasmids used in this work.

name	description
pACYC Duet-1	..
pCDF Duet-1	..
pET20b(+)	...
pET28a(+)	..
pET32a(+)	..
pET41a(+)	..
pQE30	..
pUC19	..

3.1.6 Primers

Table 3.2.: Primers used in this work. Recognition sites for endonucleases are underlined.

name	sequence (5'→3')	cloning site
T7	TAATACGACTCACTATAGGGT	n/a
T7.term	GCTAGTTATTGCTCAGCGGT	n/a
somt1	TTGAAGACAAAATGGCTTCTTCATTAAACAATGGCCG	BpiI
somt2	TTGAAGACAAGGACACCCCAAATACTGTGAGATCTTCC	BpiI
somt3	TTGAAGACAAGTCCTTAGGAACACCTTTCTGGGAC	BpiI
somt4	TTGAAGACAAAAGCTCAAGGATAGATCTCAATAAGAGAC	BpiI

3.1.7 Software

3.2 Microbiology

3.3 Molecular Biology

3.4 Protein biochemistry

3.5 Analytics

4 Evaluation of PFOMT towards the acceptance of long-chain SAM analogues

5 Enzymatic methylation of Non-catechols

testing the HPLC and again the HPLC.

Blöalala phenylpropanoid and flavonoid O-methyl transferase (PFOMT) and PFOMT

6 Development of an whole cell methyl transferase screening system

7 Acknowledgements

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III

Appendix

A Figures

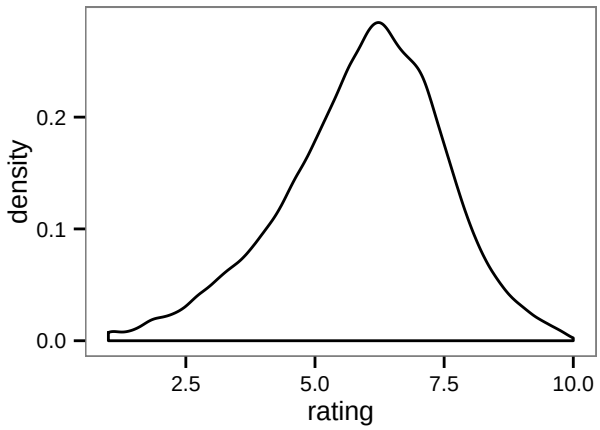

Figure A.1.: *Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Aenean commodo ligula eget dolor. Aenean massa. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Donec quam felis, ultricies nec, pellentesque eu, pretium quis, sem.*

B Tables

Table B.1.: *Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Aenean commodo ligula eget dolor. Aenean massa. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Donec quam felis, ultricies nec, pellentesque eu, pretium quis, sem.*

A	B	C	D	E	F	G	H	I
1	2	3	4	5	6	7	8	9
1	2	3	4	5	6	7	8	9
1	2	3	4	5	6	7	8	9

C Affidavit

I hereby declare that this document has been written only by the undersigned and without any assistance from third parties. Furthermore, I confirm that no sources have been used in the preparation of this document other than those indicated in the thesis itself.
Date: . . . . . , Location: . . . . . , Signature: . . . . . .

Bibliography

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Vogt, Thomas (2004). “Regiospecificity and kinetic properties of a plant natural product O-methyltransferase are determined by its N-terminal domain.” In: *FEBS letters* 561.1-3, pp. 159–62.

Acronyms

CD circulary dichroism. 10

FPLC fast protein liquid chromatography. 10

GC/MS gas chromatography coupled mass-spectrometry. 10

HPLC high-performance liquid chromatography. 10, 15

ITC Isothermal Titration Calorimetry. 10, 33

MTP micro-titer plate. 10, 33

NMR nuclear magnetic resonance. 10

PFOMT phenylpropanoid and flavonoid O-methyl transferase. 15, 33

Glossary

Isothermal Titration Calorimetry (ITC) Fill in description here. . 31

MTP Micro-titer plate. Small format rectangular plastic plate containing wells to allow for storage of multiple small samples or the containment multiple simultaneous reactions. Typical sizes include 24, 96 and 384-wells. 31

PFOMT Phenylpropanoid and flavonoid O-methyl transferase from *Mesembryanthemum crystallinum*, which was first described by Ibdah (Ibdah et al., 2003). 31