



Universiteit
Leiden
The Netherlands

Enzymology and regulation of the atropine metabolism in *pseudomonas putida*

Stevens, W.F.

Citation

Stevens, W. F. (1969, June 18). *Enzymology and regulation of the atropine metabolism in pseudomonas putida*. Retrieved from <https://hdl.handle.net/1887/77056>

Version: Not Applicable (or Unknown)

License: [Leiden University Non-exclusive license](#)

Downloaded from: <https://hdl.handle.net/1887/77056>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/77056> holds various files of this Leiden University dissertation.

Author: Stevens, W.F.

Title: Enzymology and regulation of the atropine metabolism in pseudomonas putida

Issue Date: 1969-06-18

LITERATURE – REFERENCES

- ADELBERG, E.A., *Bact. Rev.*, 17 (1953) 253
- AMMON, R. and W. SAVELSBERG, *Z. Physiol. Chem.*, 284 (1949) 135
- ASAKAWA, T., H. WADA and T. YAMANO, *Biochem. Biophys. Acta*, 170 (1968), 375
- BÄCKLIN, K.I., *Acta Chem. Scand.*, 12 (1958) 1279
- BERENDS, F., personal communication (1965)
- BERENDS, F., A. RÖRSCH, W.F. STEVENS : *Proc. Conf. Struct. Reactions DFP-sensitive enzymes*, ed. E. Heilbronn, Försvarets Forskningsanstalt Stockholm 1967 p.45.
- BERENDS, F., A.RÖRSCH, W.STEVENS, R.OOSTERBAAN, J.A.COHEN, 1969 in prep.
- BERNHEIM, F., *Interaction of Drugs and Cell Catalysts*, Burgess, Minneapolis, 1948
- BERNHEIM, F. and M.L.C. BERNHEIM, *J. Pharmacol.*, 64 (1938) 209
- CALLAHAN, J.W. and G. W. KOSICHI, *Can. J. Biochem.*, 45 (1967) 839
- CHAMBERLAIN, E.M. and S. DAGLEY, *Biochem. J.*, 110 (1968) 755
- CHAPMAN, P.J. and D.J. HOPPER, *Biochem. J.*, 110 (1968) 491
- CHILSON, O.P., G.B. KITTO, N.O. KAPLAN, *Proc. Natl. Acad. Sci. U.S.*, 53 (1965) 1006
- CIOTTI, M., N. KAPLAN, *Meth.Enzymol. III*, Colowick Kaplan, Acad. Press. NY 1957, 890
- COHEN, J.A., R.A. OOSTERBAAN, H.S. JANSZ, F. BERENDS, *J. Cell Comp. Physiol.*, 54 Suppl.1 (1959) 231
- COHEN-BAZIRE, G., W. SISTROM, R.Y. STANIER, *J. Cell. Comp. Physiol.*, 49 (1957) 25
- COMMISSION OF ENZYMES. *Rep. Intern.Union Biochem.*, Pergamon, Oxford, 1961 p. 7
- COZZARELLI, N., S. HAYAISHI and E.C.C. LIN, *Federation Proc.*, 24 (1965) 417
- DAGLEY, S. et al, *Pseudomonas Workshop*, Cold Spr. Harb. Quant. Biol., NY, 1964, p.7.
- DAVIES, M. and W.J. ORVILLE-THOMAS, *J. Chem Soc.*, (1951) 2858
- FLEISCHMAN, P., *Berl. Klin Wschr.*, 48 (1910) 135
- FLODIN, P. and D.W. KUPLIE, *Biochim. Biophys. Acta*, 21 (1956) 368
- FLUCK H., *Pharm. Weekblad*, 100 (1965) 1361
- FRIEDMAN, C. A. and J.M.Z. GLADYCH, *J. Chem. Soc.*, (1956) 310
- GABOUREL, J.D. and R.E. GOSSELIN, *Arch. Intern. Pharmacodyn.*, 115 (1958) 416

GIBSON, C.A. and H.W. YOUNGKEN, J. Pharm. Sci., 56 (1967) 854

GIBSON, D.T., Science, 161 (1968) 1093

GLICK, D., J. Biol. Chem., 134 (1940) 617

GUNSALUS, C.F., R.Y. STANIER and I.C. GUNSALUS, J. Bacteriol., 66 (1953 a) 548

GUNSALUS, I.C., C.F. GUNSALUS and R.Y. STANIER, J. Bacteriol., 66 (1953 b) 538

HEGEMAN, G.D., J. Bacteriol., 91 (1966) 1140

HOLLOWAY, B., P. VAN DE PUTTE, Nature, 217 (1968) 459

HOUBEN-WEIJL, Method. Organischen.Chem. Bd II Thieme, Stuttgart, (1953) p. 385

JANSON, J., J. Chromat., 28 (1967) 12

JINDRA, A., A. CIHAK, Abh. Deutch, Akad. Wiss., Chem. Geol. Biol, Berlin, 1963 nr 4

KACZKOWSKI, J., Acta Soc. Botan. Polon., 28 (1959) 677

KACZKOWSKI, J., Bull. Acad. Polon. Sci. Ser. Sci. Biol., 12 (1964) 375

KALSER, S.C., et al., J. Pharmacol. Exp. Therap., 121 (1957) 449

KAUFMAN, H.P. and E. RICHTER, Chem. Ber., 58 (1925) 222

KEDZIA, W., J. LEWON and T. WISNIEUWSKI, J. Pharm. Pharmacol., 13 (1961) 614

KENNEDY, S.I.T. and C.A. FEWSON, Biochem. J., 107 (1968) 497

KOHN, L.D., and W.B. JACOBY, J. Biol.Chem., 243 (1968) 2465

KRUPKA, R.M., Biochemistry, 2 (1963) 76

KUBOWITZ, F. and P. OTT, Biochem. Z., 314 (1943) 94

LAYNE, E., in: Meth. Enzymol. III, ed. Colowick Kaplan, Acad. Press, NY, (1957) p. 447

LEDERBERG, J. and E.M.LEDERBERG, J. Bacteriol., 63 (1952) 399

LINDEN, A.C, VAN DER and G.J.E.THIJSSE, Advances in Enzymology, 27 (1965) 469

LINEWEAVER, H. and D. BURK, J. Amer. Chem Soc., 56 (1934) 658

LINN, S. and I.R. LEHMAN, J. Biol. Chem., 240 (1965) 1287

LOEWUS, F.A., T.T. CHEN and B. VENNESLAND, J. Biol. Chem., 212 (1955) 787

LOWRY, O., N. ROSEBROUGH, A. FARR, R. RANDALL, J.Biol.Chem. 193 (1951) 265

MARGOLIS, F. and P. FEIGELSON, J. Biol. Chem., 238 (1963) 2620

MARGOLIS, F. and P. FEIGELSON, Biochem.Biophys. Acta, 90 (1964) 117

MATSUDA, K., Niigata Igakkai Zasshi, 80 (1966) 53

METZNER, R., Arch. Exp. Pathol. Pharmacol., 68 (1912) 110

MOORE, C.L., Arch. Biochem. Biophys., 128 (1968) 734

MÜLLER-HILL, B., H. RICKENBERG K. WALLENFELS, J. Mol. Biol., 10 (1964) 303

NEUMANN, D. and K. H. TSCHÖPE, Flora Jena, 156 (1966) 521

NIEMER, H., H. BUCHERER and A. KOHLER, Z. Physiol. Chem., 317 (1959) 238

NIEMER, H. and H. BUCHERER, Z. Physiol. Chem., 326 (1961) 9

ORNSTON, L.N., J. Biol Chem., 241 (1966) 3800

OTORII, T., Nippon Yakurigaku Zasshi, 61 (1965) 462

PARDEE, A.B., Science, 162 (1968) 632

QUINTON, R.M., J. Pharm. Pharmacol., 18 (1966) 579

RÖRSCH, A. and F. BERENDS, personal communication (1965)

RODD, E.H., Chem. Carbon Comp. Vol. III B, Elsevier, Amsterdam , 1956 p. 927

SAWIN, P.S. and D. GLICK, Proc. Natl. Acad. Sci. U.S., 29 (1943) 55

SCHACHMAN, H. K., Cold Spr. Harb. Symp. Quant. Biol., 28 (1963) 409

SCHLESINGER, S. and B. MAGASANIK, J. Biol. Chem., 249 (1965 a) 4325

SCHLESINGER, S., P. SCOTTO and B. MAGASANIK, J. Biol. Chem. 240 (1965 b) 4331

SCHROFF, Z., Z. Ges. Ärzte Wien, 3 (1852) 211

SEIDENBERG, M. et al, Arch. Biochem. Biophys., 97 (1962) 470

STACHOW, C.S., I.L. STEVENSON and D. DAY, J. Biol. Chem., 242 (1957) 5294

STAHL, E., Dünnschicht Chromatographie, Springer Verlag, Berlin, 1957.

STANIER, R.Y., Bact. Rev., 14 (1950) 179

STANIER, R.Y., I.C.GUNSALUS and C.F.GUNSALUS, J. Bacteriol. , 66 (1953) 543

STANIER, R.Y., N.J. PALLERONI, M. DOUDOROFF, J. Gen. Microbiol, 43 (1966) 159

STRUKOV, I.T., Zhur Obschei Khim., 22 (1952) 1025

SUBBA RAO, P.V., K. MOORE, G.TOWERS, Arch. Biochem. Biophys., 122 (1967) 466

UMBREIT, W.W. Manometric Techn.Tissue Metabol., Burgess, Minneapolis, 1951

VOGEL, A.I., Practical Organic Chemistry, Longmans, London, 1959, p. 781

WENSINCK, F., Appendix at Berends (1969)

WERNER, G. and G. BREHMER, Z. Phys. Chem. , 348 (1967) 1640

WERNER, G. and K.H. SCHMIDT, Z. Physiol Chem., 349 (1968) 741

OVERVIEW OF THE ACADEMIC CAREER OF WILLEM FRANS STEVENS

Presented here at the request of the Faculty of Science,
State University Leyden, The Netherlands.

After graduation in 1959 at the Grammar School β -division of the Twentsch Carmel Lyceum in Oldenzaal, The Netherlands, I was enrolled in the same year for a study in chemistry at the State University Leyden. In 1962, I passed the bachelors exam and in 1965 the master's exam with honors in the main discipline of biochemistry and in organic chemistry and pharmacology, under guidance of Prof. Dr. L. Bosch, Prof. Dr. E. Havinga and Prof. Dr. E. L. Noach.

To fulfill my duties in military service I followed a curtailed training for army officer. Thereafter, I was sent on secondment to the Medical Biological Laboratory (MBL) of the National Defense Organization TNO Rijswijk, The Netherlands. I got permission to make a start with the research presented in this thesis. After fulfillment of my military duties, I was engaged by the State University Leyden as co-worker of the Institute for Radiopathology and Irradiation Protection, but remained employed at the MBL in order to continue the research on the regulation of enzyme synthesis in *Pseudomonas*.

It is a deep honor to thank my PhD promoter Prof. Dr. A. Rörsch and Prof. Dr. J.A. Cohen, director of the MBL for their contributions to my academic education.

Especially, I am indebted to Dr. F. Berends for his thorough and critical interest and his comments regarding the editing of the Dutch manuscript, to Ir. C. A. van Sluis for his great interpretation of the concept of good-fellowship and to Dr. R. A. Oosterbaan for his valuable advices.

Further I acknowledge many co-workers of the MBL and the Chemical Laboratory of the National Defense Organization TNO who contributed to the research presented in this thesis. I especially name Miss M. P. van Winden for the quick and accurate assistance during 4 years and Mrs T. Roodenhuis-Poelman and Mr. J. A. M. Glandorf for their contribution in the experimental work, Mr H. E. Groot Bramel, M. J. J. Boermans and J. J. Weber for figures and photos and Miss E. Looy for secretarial assistance.

The Board of the National Defense Research Organization TNO I am indebted for the opportunity to do this thesis project in the MBL.

Finally, I thank you, Yvonne, for your stimulus and practical assistance and You, my parents for all You did to give me the academic training that culminates in this PhD graduation.

ABBREVIATIONS

MBL	Medical Biological Laboratory
TNO	Dutch Organization for Applied Scientific Research
PMBL-1	Pseudomonas bacterium isolated from Atropa belladonna soil
Ps-atropine	PMBL-1 grown in the presence of atropine
Ps-tropic acid	PMBL-1 grown in the presence of tropic acid

AtrE	atropine esterase
TDH	tropic acid dehydrogenase
PDC	2-phenylmalonic semi-aldehyde decarboxylase
PDH	phenylacetaldehyde dehydrogenase
NAD ⁺	nicotinamide adenine dinucleotide
NADH	reduced NAD ⁺
NADP ⁺	nicotinamide adenine dinucleotide phosphate
NADPH	reduced NADP ⁺
pma	2-phenylmalonic semi-aldehyde
enol-pma	tautomeric enol-form of pma
keto-pma	tautomeric keto-form of pma

nm	nanometer
A ₃₄₀	absorption at 340 nm
A ₇₀₀	absorption at 700 nm
U	unit of enzyme activity
TA	total activity
SA	specific activity

HMP	10 mM K-phosphate buffer pH 7.0
EDTA	ethyleendiamino tetra-acetic acid
ME	mercapto ethanol
SDS	sodium lauryl sulfate
TRIS	tris hydroxymethyl aminomethane
LDH	lactic acid dehydrogenase

GENETIC MARKERS

Atr	atropine
Tro	tropic acid
Tpn	tropine
Pac	phenylacetic acid
Php	p-hydroxylphenylacetic acid
Pgl	phenylglyoxylic acid
AtrE-	mutant lacking the AtrE

The relation between the amount or concentration and the absorption at 340 nm in a volume of 3 ml: 1 μ mol NADH $\equiv$ A₃₄₀ 2.07 ; 1 mM NADH $\equiv$ A₃₄₀ 6.22

CONTENTS

INTRODUCTION		7
CHAPTER 1 LITERATURE		13
1.1	Breakdown of atropine in mammals	13
1.2	Breakdown of atropine in plants	15
1.3	Breakdown of atropine in micro-organisms	15
CHAPTER 2 MATERIALS AND METHODS		
2.1	Nomenclature	12
2.2	Materials	17
2.3	Isolation of <i>Pseudomonas</i> PMBL-1	18
2.4	Cultivation of the bacteria	19
2.5	Isolation of <i>Pseudomonas</i> mutants	20
2.6	Quantification of oxygen consumption and CO ₂ production	21
2.7	Thin Layer Chromatography	22
2.8	Isolation of ³ H tropic acid, uptake of ³ H tropic acid in <i>Pseudomonas</i>	23
2.9	Assay of enzyme activities	24
2.9.1	General	24
2.9.2	Quantitative assay of AtrE activity	24
2.9.3	Quantitative assay of TDH-, PDC- and PDH-activity	25
2.10	Assay of specific enzyme activity in extracts of PMBL-1 and mutants	27
2.11	Purification of the tropic acid enzymes	29
2.11.1	Purification of AtrE	29
2.11.2	Purification of TDH, PDC and PDH	31
2.11.3	Estimation of the molecular weight of the tropic acid enzymes	31
2.12	Spectroscopic and chemical analysis	33
2.13	Assay of the enolic content of 2-phenylmalonic semi-aldehyde	34
CHAPTER 3 SYNTHESIS AND PROPERTIES OF 2-PHENYLMALONIC SEMI-ALDEHYDE		
3.1	Introduction	35
3.2	Synthesis of 2-phenylmalonic semi-aldehyde	36
3.3	Identification of 2-phenylmalonic semi-aldehyde	37
3.3.1	Range of the thermal decomposition	37
3.3.2	Infrared and Nuclear Magnetic Resonance spectroscopy	38
3.3.3	Gas Liquid Chromatography	39
3.3.4	Biochemical data	39

3.4	Tautomeric rearrangement of the 2 phenylmalonic semi-aldehyde	39
3.4.1	Quantitative assay of the enolic form	39
3.4.2	Shift of the keto-enol equilibrium in ethanol at 0°	40
3.4.3	Rate of the tautomeric rearrangement in aqueous solution	42

CHAPTER 4 BREAKDOWN OF ATROPINE AND TROPIC ACID IN PSEUDOMONAS PMBL-1

4.1	Introduction	43
4.2	Carbon sources for PMBL-1	44
4.3	The adaptation of PMBL-1 to aromatic acids	45
4.4	Investigation of mutants, blocked in the breakdown of tropic acid	47
4.5	Mutants blocked in the metabolism of phenylacetic acid and p-hydroxyphenylacetic acid	48
4.6	Investigation mutants blocked in the metabolism of phenylacetic acid	49
4.7	Breakdown of phenylacetic acid	51
4.8	Discussion	51

CHAPTER 5 THE ATROPINE ESTERASE

5.1	Introduction	53
5.2	Quantitative assay of the activity of the atropine esterase	53
5.3	Purification of the atropine esterase	54
5.4	Properties of the atropine esterase	55
5.4.1	Analysis of the products of the enzyme reaction	55
5.4.2.	Stoichiometry	55
5.4.3	Substrate optimum	55
5.4.4	pH optimum	58
5.4.5	Substrate specificity	58
5.4.6	Stereo specificity	60
5.4.7	Stability of the enzyme	60
5.4.8	Miscellaneous	61
5.5	Discussion	61

CHAPTER 6 THE TROPIC ACID DEHYDROGENASE

6.1	Introduction	64
6.2	Quantitative assay of the tropic acid dehydrogenase	66
6.3	Partial purification	67
6.4	Properties of the purified enzyme	67
6.4.1	Analysis of the reaction products and stoichiometry of the reaction	67

6.4.2.	Effect of pH on enzymatic dehydrogenation	69
6.4.3	Specificity of the tropic acid dehydrogenase	70
6.4.4.	Stability	72
6.4.5	Some other features	72
6.5	Enzymatic dehydrogenation of tropic acid in neutral environment	73
6.6	The effect of the keto-enol tautomeric rearrangement on the enzymatic hydrogenation of pma	80
6.7	The speed of the spontaneous decomposition of pma	86
6.8	The effect of the keto-pma on the establishment	89
6.9	Discussion	90

CHAPTER 7 THE 2-PHENYLMALONIC SEMI-ALDEHYDE DECARBOXYLASE

7.1	Introduction	94
7.2	Indications for the presence of a decarboxylase	94
7.3	Quantitative assessment of the PDC activity	101
7.4	Partial purification of PDC	101
7.5	Properties of the purified enzyme	102
7.5.1	Substrate specificity	102
7.5.2	Specificity for the keto-form of pma	103
7.5.3	The stability of the enzyme	104
7.5.4	Miscellaneous	104
7.6	Discussion	105

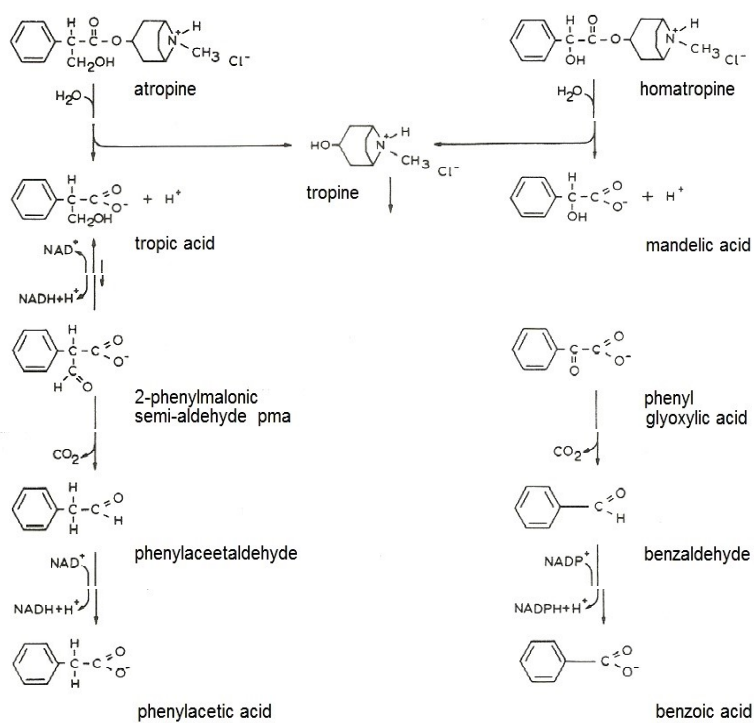
CHAPTER 8 THE PHENYLACETALDEHYDE DEHYDROGENASE

8.1	Introduction	107
8.2	Assay and stability of PDH	107
8.2.1	Stability during dialysis	108
8.2.2	Stability in diluted samples	109
8.3	Partial purification of PDH	110
8.4	Some properties of the purified enzyme	111
8.4.1	Analysis of the products of the enzymatic conversion	111
8.4.2	Stoichiometry of the reaction	111
8.4.3	The pH optimum	111
8.4.4	Specificity of PDH	112
8.5	Conversion of pma by a partially purified PDH sample	114
8.6	Miscellaneous	116
8.7	Discussion	118

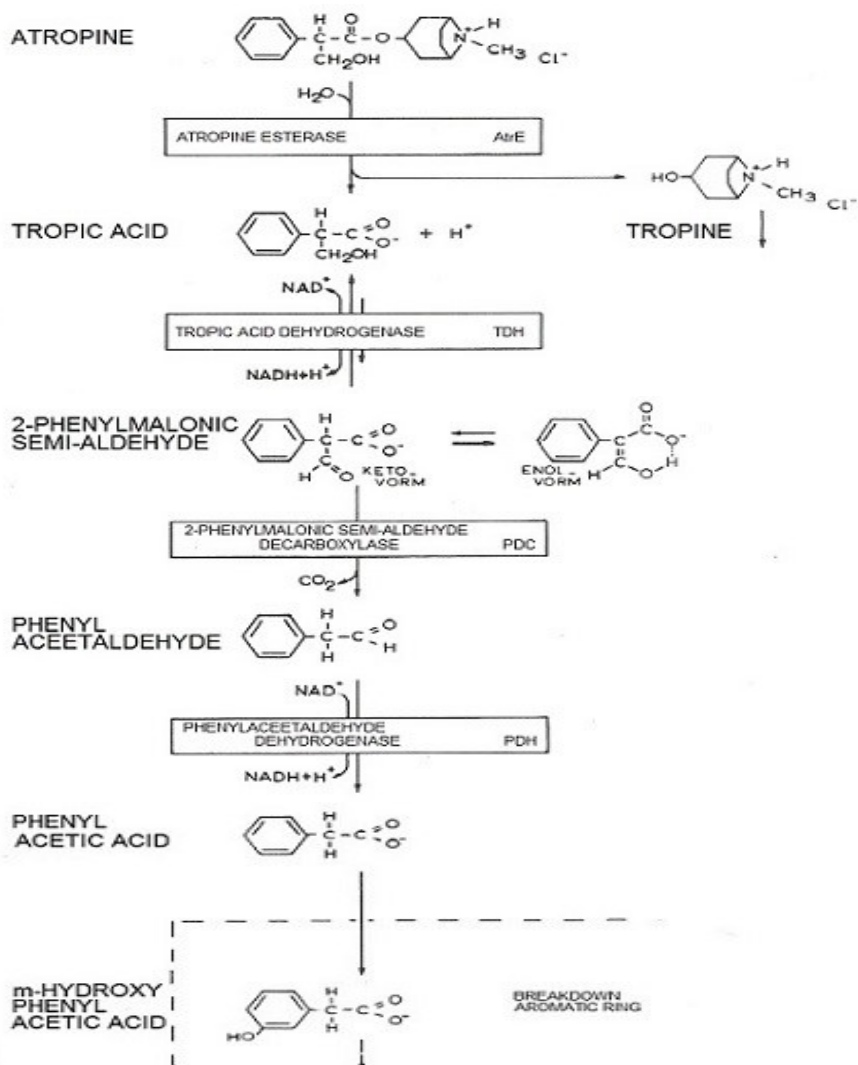
CHAPTER 9	THE TROPIC ACID ENZYMES	
9.1	Introduction	119
9.2	Sequence of action by the tropic acid enzymes	119
9.3	Other enzymes that might be involved in the breakdown of atropine	121
9.3.1	Breakdown of tropine	121
9.3.2	Uptake of substrates from the medium	121
9.3.3	Racemase	123
9.4	Breakdown of atropine in other Pseudomonaceae	123
9.5	Relation with metabolism of mandelic acid and phenylpyruvic acid	125
CHAPTER 10	REGULATION OF THE SYNTHESIS OF THE TROPIC ACID ENZYMES	
10.1	Introduction	128
10.2	Kinetics of induction; the effect of chloramphenicol	130
10.3	The specificity of the induction of tropic acid enzymes	132
10.4	Gratuitous induction by tropic acid and phenylacetaldehyde	134
10.5	Gratuitous induction by phenylglyoxylic acid and benzaldehyde	135
10.6	Induction in mutants of PMBL-1	136
10.7	Overview of the PMBL-1 mutants used	139
10.8	Discussion	140
SUMMARY IN DUTCH LANGUAGE		144
SUMMARY		149
LITERATURE		152
CV AND AKNOWLEDGEMENTS		155
ABBREVIATIONS		156
TABLE OF CONTENTS		157
ANNEX : PATHWAYS FOR ATROPINE AND PHENYLGLYOXYLIC ACID IN PSEUDOMONAS PUTIDA PMBL-1		161
TROPIC ACID ENZYMES INVOLVED IN THE METABOLISM OF ATROPINE IN PSEUDOMONAS PML-1		163
THESES	ENCLOSURE	
EPILOGUE	ENCLOSURE	

Annex

PATHWAYS FOR ATROPINE AND PHENYLGLYOXYLIC ACID IN PSEUDOMONAS PMBL-1



**TROPIC ACID ENZYMES :
INVOLVED IN THE METABOLISM OF
ATROPINE IN PSEUDOMONAS PUTIDA PMBL-1**



Author: Prof. Dr. Willem Frans Stevens

© <wffstevens@gmail.com

Cover design: kirsten, ivo@bravenewbooks.nl

ISBN: 9789 4021 91 448

PRINTING DATA

1969: Dutch version

Composer and typesetting: Zetterijbedrijf Sannen,
Rijswijk ZH The Netherlands

Offsetprint: Demmenie N.V. Leyden The Netherlands

2019 English version

Publisher Ivo Bravenewbooks.

The English version is a precise translation of the Dutch version 1969. No update.