# Enzymology and regulation of the atropine metabolism in pseudomonas putida

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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.

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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 aid |
| | |
| 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

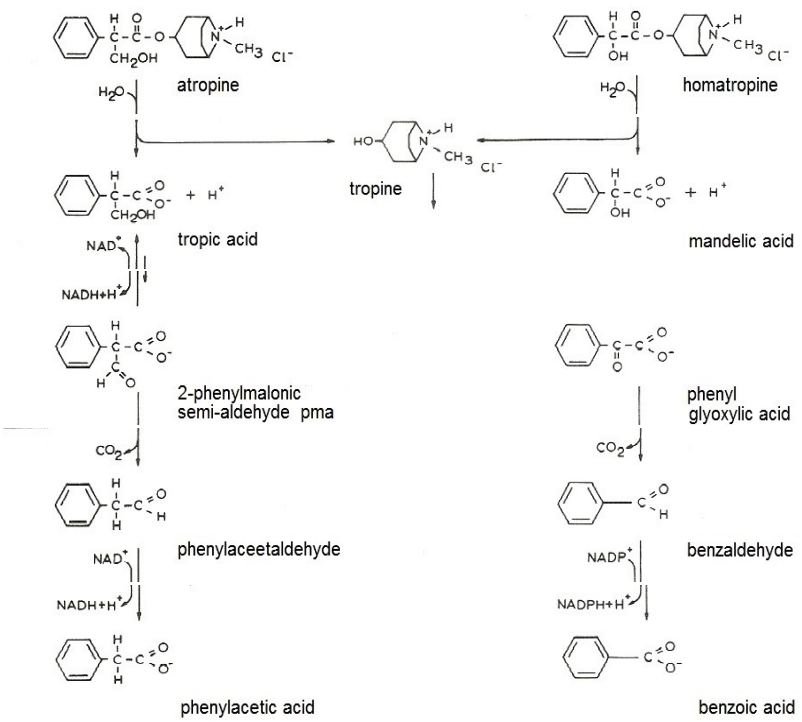
| | |
|---|---|
| Atr | atropine |
| Tro | tropic acid |
| Tpn | tropine |
| Pac | phenylacetic acid |
| Php | p-hydroxylphenylacetic acid |
| Pgl | phenylglyoxylic acid |
| AtrE- | mutant lacking the AtrE |

The relation between the amount or concentration and the absorption at 340 nm in a volume of 3 ml: 1 µmol NADH ≡ $A_{340}$ 2.07 ; 1 mM NADH ≡ $A_{340}$ 6.22

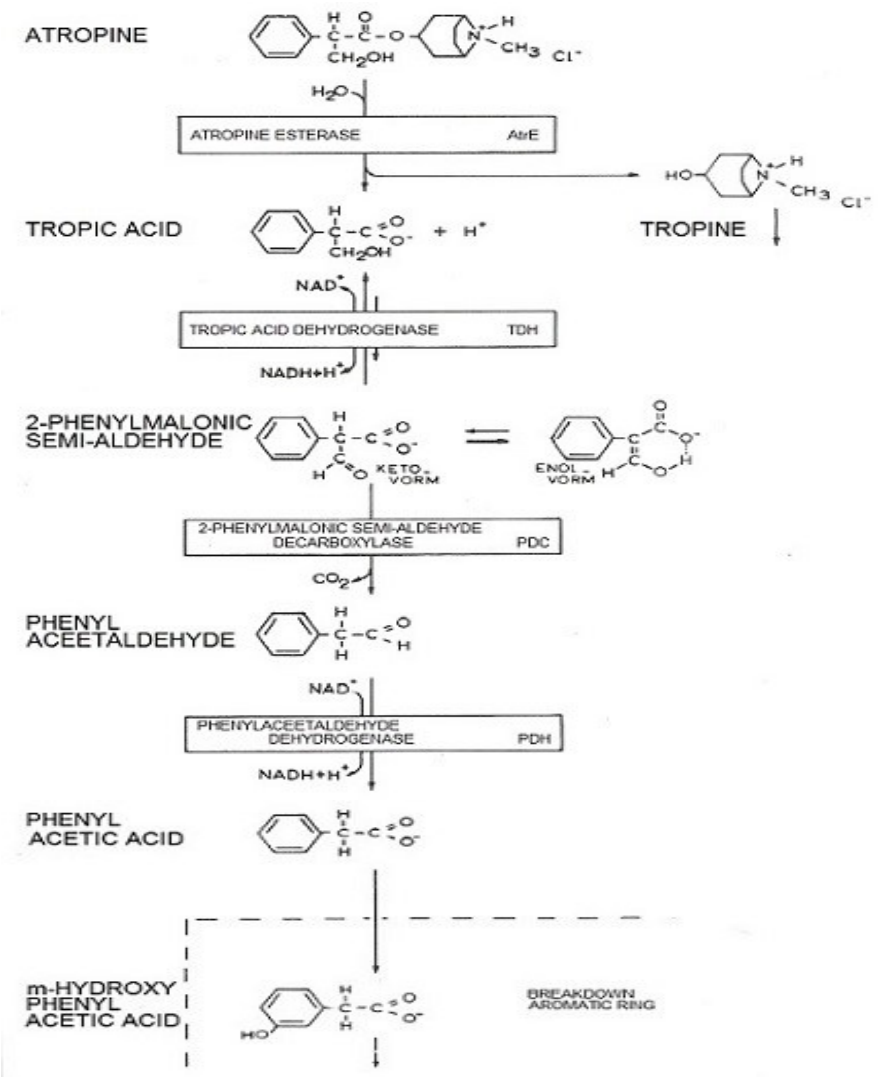
CONTENTS

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



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