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

Stevens, W.F.

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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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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-hydroxyphenylacetic 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

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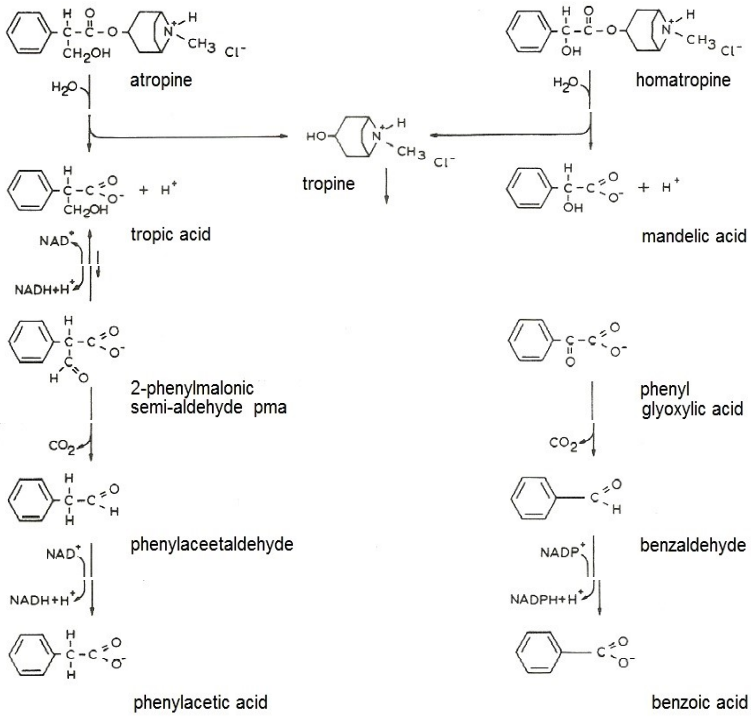
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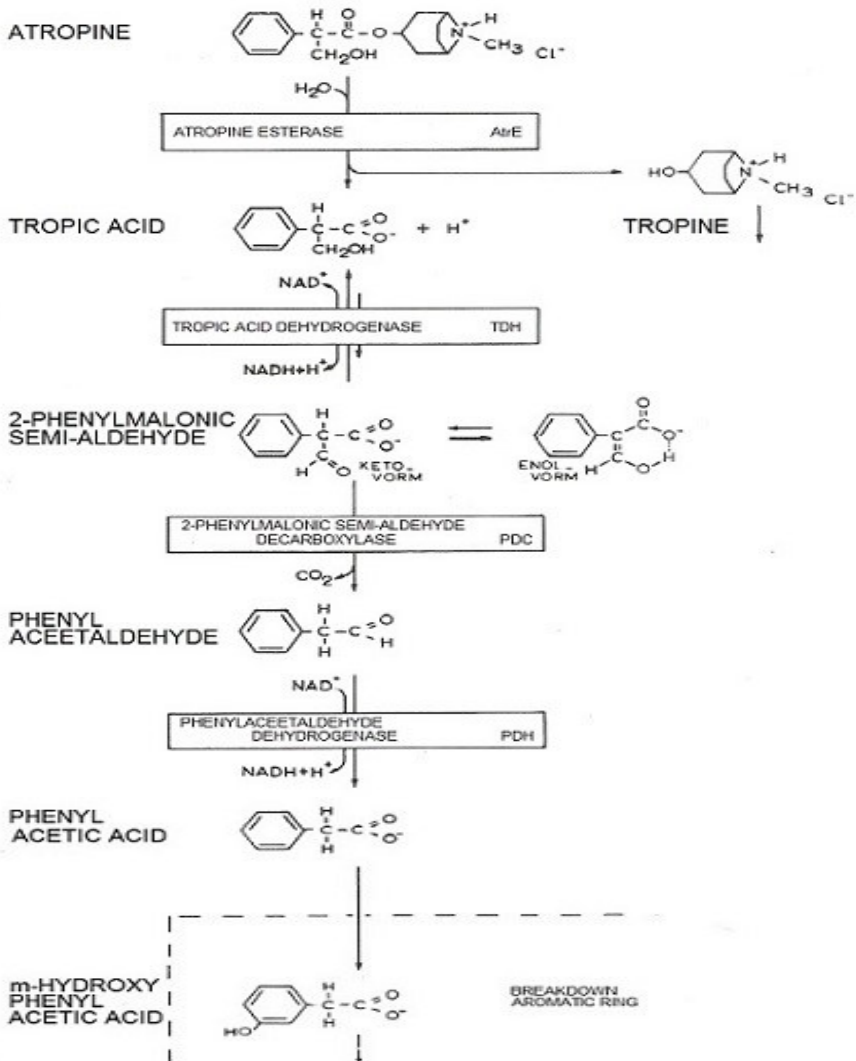
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Annex

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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