



A study of  $\alpha$ -aminobutyronitrile in *Rhizoctonia solani*  
by Francis Hing Soon Liu

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of  
MASTER OF SCIENCE in Chemistry  
Montana State University  
© Copyright by Francis Hing Soon Liu (1972)

Abstract:

$\alpha$ -aminobutyronitrile was found in *Rhizoctonia solani* as a product of potassium cyanide assimilation.  
Labeled potassium cyanide ( $\text{KC}^*\text{N}$ ) was used to study the pathways.

Statement of Permission to Copy

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at Montana State University, I agree that the Library shall make it freely available for inspection. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by my major professor, or, in his absence, by the Director of Libraries. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature

James L. K. Sood

Date

Aug 8, 1972

A STUDY OF  $\alpha$ -AMINOBUTYRONITRILE  
IN RHIZOCTONIA SOLANI

by

FRANCIS HING SOON LIU

A thesis submitted to the Graduate Faculty in partial  
fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

Chemistry

Approved:

*E. W. Quaker*

Head, Major Department

*Bradford Philip Mundy*

Chairman, Examining Committee

*K. Goering*

Graduate Dean

MONTANA STATE UNIVERSITY  
Bozeman, Montana

August, 1972

## ACKNOWLEDGMENT

I would like to first acknowledge the Endowment and Research Foundation of Montana State University for its support of Dr. B. P. Mundy's research endeavors. I would also like to thank the Department of Chemistry at Montana State University for their financial support in the way of teaching assistantships and tuition waivers. I would like to thank Dr. Mundy for his continued help and interest not only in the area of my research, but also the overall value of my education at this institution. Without his patience and confidence, this thesis would not have been possible. I would also like to acknowledge the help of the staff members and graduate students of the department for their discussion and technical aid to my special problems, especially Dr. K. Hapner for the assistance in setting up and operating the amino acid analyzer; Drs. S. Roger and G. Julian in using the electrophoresis instrument; Dr. L. Jackson in preparing TLC plates and in the use of scintillation counting, and Dr. P. Jennings for mass spectrometry.

Especially, I would like to thank Dr. G. A. Strobel for all the help and suggestions he gave throughout the

course of this research. I would like to thank my colleagues in our research group, Mr. B. Larsen and Mr. J. Sun for their meaningful discussion.

Most importantly, I would like to thank my parents, without whom all of this would not have been possible.

## TABLE OF CONTENTS

	Page
VITA . . . . .	ii
ACKNOWLEDGMENT . . . . .	iii
TABLE OF CONTENTS . . . . .	iv
LIST OF TABLES . . . . .	vi
LIST OF FIGURES . . . . .	vii
ABSTRACT . . . . .	ix
INTRODUCTION . . . . .	1
DISCUSSION AND RESULTS . . . . .	8
Synthesis of $\alpha$ -aminonitrile . . . . .	8
Fungus Selection and Culture . . . . .	11
Incorporation of Potassium Cyanide . . . . .	13
Reaction of $\alpha$ -aminobutyronitrile . . . . .	17
CONCLUSION . . . . .	23
METHODOLOGY . . . . .	27
Enzymes Extract . . . . .	27
Amino Acid Analyzer . . . . .	30
Radioisotopic Technique . . . . .	34
EXPERIMENTAL . . . . .	35
Solvents for Thin Layer Chromatography . . . . .	35
Buffer System for Electrophoresis . . . . .	36

	Page
Synthesis of $\alpha$ -aminonitrile by Teimann's Method . . . . .	36
Preparation of $\alpha$ -aminoacetonitrile . . . . .	36
Preparation of $\alpha$ -aminopropionitrile . . . . .	37
Synthesis of $\alpha$ -aminonitrile with Lowy's Method . . . . .	37
Preparation of $\alpha$ -aminobutyronitrile . . . . .	38
Enzymatic Reactions . . . . .	39
Reaction with Enzymes Extract . . . . .	39
Reaction with Whole Fungus . . . . .	40
Reaction with Homogenized Fungus . . . . .	40
Medium to <u>R. solani</u> . . . . .	41
REFERENCES . . . . .	42

## LIST OF TABLES

Table	Page
1. Data From Two Identical Samples Using Different Solvents on TLC . . . . .	16
2. Data From Two Identical Samples Using Different Solvents on Two Dimensional TLC . . . . .	17
3. Data For Conversion of $\alpha$ -aminobutyronitrile to $\alpha$ -aminobutyric Acid . . . . .	18
4. Data From Carbon Dioxide Reaction . . . . .	19
5. Graph of $\alpha$ -aminobutyronitrile and $\alpha$ -aminobutyric acid vs time . . . . .	22



## LIST OF FIGURES

Figure	Page
1. Proposed Scheme of HCN Assimilation . . . . .	1
2. Scheme of Incorporation of Cyanide to Aspartic Acid . . . . .	2
3. Scheme of Incorporation of Cyanide in a Fungus . . . . .	3
4. Proposed Mechanism of Nitrilase . . . . .	4
5. Decarboxylase Reaction . . . . .	5
6. Strecker's Synthesis of $\alpha$ -aminonitriles . . . . .	6
7. Mechanism of Ressler's Synthesis . . . . .	6
8. Proposed Mechanism of Strecker's Synthesis of $\alpha$ -aminonitrile . . . . .	8
9. Proposed Mechanism of Tiemann's Synthesis of $\alpha$ -aminonitrile . . . . .	9
10. Proposed Path of Enzymatic Conversion of Propanal to $\alpha$ -aminoacid . . . . .	13
11. Schematic Procedure for Dead Fungus Experiment . . . . .	15
12. Typical TLC Plate . . . . .	21
13. Typical Two Dimensional TLC Plate . . . . .	21
14. Proposed KCN Pathway Yielding $\alpha$ -aminobutyric Acid . . . . .	24
15. Scheme for Extraction of Enzymes . . . . .	29
16. Schematics of Beckmann Amino Acid Analyzer . . . . .	32

Figure	Page
17. Schematic of Technicon Autoanalyzer . . . . .	33

ABSTRACT

$\alpha$ -aminobutyronitrile was found in Rhizoctonia solani as a product of potassium cyanide assimilation. Labeled potassium cyanide (KC\*N) was used to study the pathways.

## INTRODUCTION

Cyanide assimilation occurs in various living systems. Some species of insects are known to be able to assimilate hydrogen cyanide and transform it into aspartic acid.<sup>1</sup> In some higher plants, it has been observed with feeding experiments that hydrogen cyanide was incorporated into asparagine to a considerable extent.<sup>2</sup> Other investigations have concluded that  $\beta$ -cyanoalanine is the intermediate of cyanide assimilation.<sup>3</sup> It has been proposed that hydrogen cyanide condenses with cysteine in the presence of an enzyme, cyanoalanine synthetase,<sup>4</sup> to produce  $\beta$ -cyanoalanine and hydrogen sulfide. This  $\beta$ -cyanoalanine can then be hydrolyzed into asparagine with a nitrilase or

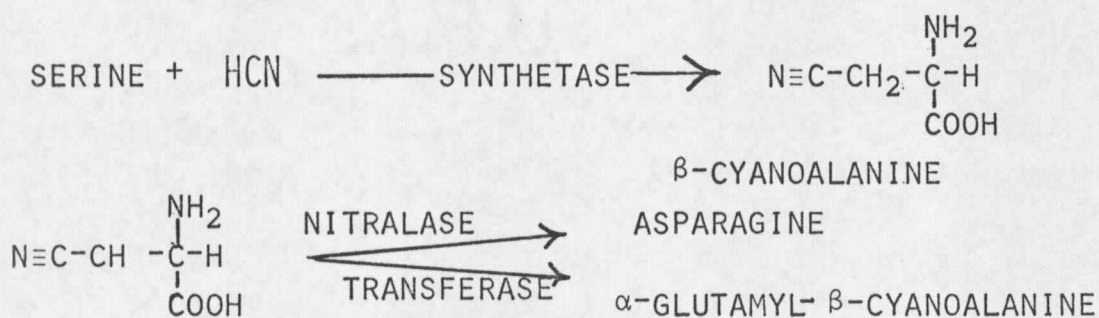


Fig. 1

Proposed Scheme of HCN Assimilation

condenses with  $\gamma$ -glutamyl moiety by means of a  $\gamma$ -glutamyl transferring enzyme to produce  $\gamma$ -glutamyl- $\beta$ -cyanoalanine.<sup>5</sup>

Castric and Strobel<sup>6</sup> reported, in a similar study, evidence of the formation of asparagine and aspartic acid from cyanide and serine by a bacterium.

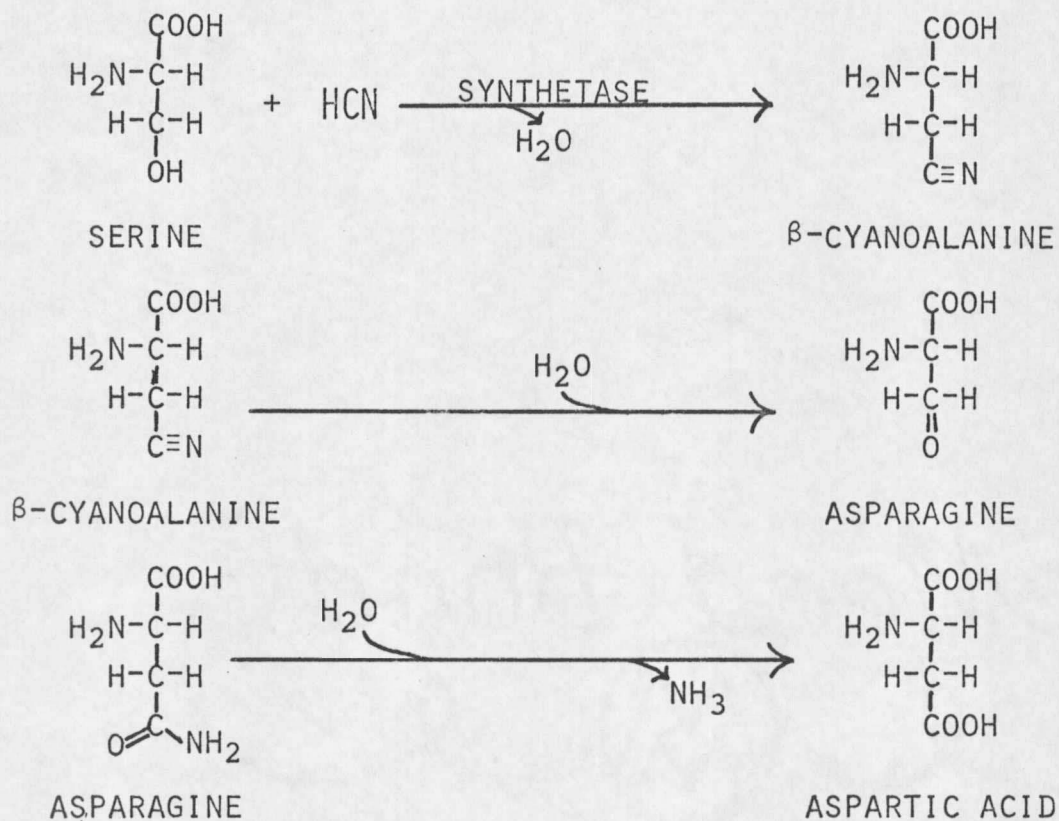


Fig. 2

Scheme of Incorporation of Cyanide to Aspartic Acid





























































































