INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

Xerox University Microfilms
300 North Zeeb Road
Ann Arbor, Michigan 48106

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

76-16,898

CLOUGH, Fred Wayne, 1949-
STUDIES INVOLVING THIENOPYRIDINE AND THIENOPYRAZINE ANALOGS OF BIOLOGICALLY ACTIVE MOLECULES.

University of South Florida, Ph.D., 1976
Chemistry, organic

Xerox University Microfilms, Ann Arbor, Michigan 48106

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

STUDIES INVOLVING THIENOPYRIDINE AND THIENOPYRAZINE ANALOGS OF BIOLOGICALLY ACTIVE MOLECULES

by

FRED WAYNE CLOUGH

Submitted to the Faculty of the Graduate School
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in the Department of Chemistry of
the University of South Florida

March, 1976

Tampa, Florida

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Graduate Council
University of South Florida
Tampa, Florida

CERTIFICATE OF APPROVAL

Ph.D. THESIS

This is to certify that the Ph.D. thesis of
FRED WAYNE CLOUGH
with a major in CHEMISTRY has been approved by the Examining Committee as satisfactory for the Ph.D. degree at the convocation of March, 1976.

Stewart W. Schneller

Dr. Stewart W. Schneller, Major Professor

Jack E. Fernandez

Dr. Jack E. Fernandez

George R. Jurch, Jr.

Dr. George R. Jurch

Jay H. Worrell

Dr. Jay H. Worrell

Janice O. Tsokos

Dr. Janice O. Tsokos

Joseph F. Williams

Dr. Joseph F. Williams, Examination Chairman

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

DEDICATION

TO CINDY who has endured many sacrifices during the last four years without complaint and whose continuous encouragement and love has been a source of inspiration and motivation,

TO DEREK who is able to dissipate the misery of a day full of intractable tars with a hug and a kiss,

TO MOM and DAD whose love has helped to make this thesis a reality and for whom this thesis means a great deal.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ACKNOWLEDGEMENTS

Learning is the greatest adventure in life. The author wishes to express his appreciation to Dr. S.W. Schneller for his encouragement and assistance in this episode of the author's development and for making this learning process stimulating and joyous. It is a testimonial to Dr. Schneller's effectiveness as a professor that he is able to transmit to his students the enthusiasm with which he approaches both life and chemistry.

The author wishes also to express his gratitude to Dr. Per Njal Skancke of the Institute of Mathematical and Physical Sciences, Department of Chemistry, University of Tromsø, who, without restrictions, provided the molecular orbital calculations presented in this thesis.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE OF CONTENTS

	Page
ABSTRACT	xii
PREFACE	xiv
INTRODUCTION	1
BIOLOGICAL BACKGROUND	17
I. Serotonin	17
II. Chemotherapeutic Antifolates	26
A. Antimalarial Agents	31
B. Antineoplastic Agents	42
CHEMICAL BACKGROUND	47
I. Thienopyridines	47
II. Thienopyrimidines	55
III. Thienopyrazines	57
DISCUSSION OF RESULTS	60
I. Synthetic Approaches to Thieno[3,2-c]-pyridines and Analogs of Serotonin	60

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Page

II. Synthetic Approaches to Thienopyridine Analogs of Antifolate Antimalarials..... 72

III. Synthetic Approaches to Thieno-Separated Analogs of Folic Acid 78

EXPERIMENTAL 91

SELECTED SPECTRA 141

REFERENCES 160

APPENDIX: Molecular Orbital Studies of the Thienopyrazines 170

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF TABLES

Number		Page
1	Grimm's Hydride Displacement Law	15
2	Relative Activities of Some Tryptamine and 5-Hydroxytryptamine Derivatives	18
3	Relative Affinities and Intrinsic Activities of Substituted Tryptamines and Their Indene Isosteres	20
4	Tetrahydrofolic Acid Coenzymes	28
5	Reactivity Indicies for Compounds 86 and 87	163
6	Comparison of Calculated Bond Distances for Compounds 75, 76, 79, 86, and 87	164

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF CHARTS

Number		Page
I	Molecular Modification of the Sulfanilamide Structure	12
II	Biosynthesis of Serotonin	24
III	*de novo* Synthesis of Folic Acid from GTP	27
IV	Biological Utilization of Tetrahydrofolic Acid Coenzymes	30
V	Molecular Framework of Separated Folic Acid Analogs	45
VI	Structures of the Isomeric Thienopyridines	48
VII	Synthesis of 3-Methylthieno[3,2-c]pyridine	63
VIII	Reissert Reaction on Thieno[3,2-c]pyridine	67
IX	Reactions of Compound 129	74
X	Synthesis of 3-Hydroxythieno[2,3-b]pyridine	77
XI	Synthesis of 2,4-Diamino-pyrido[3',2':4,5]thieno[3,2-d]pyrimidine	77
XII	Synthesis of Pyrazino[2',3':4,5]thieno[3,2-d]pyrimidine	81
XIII	Reactions of Compound 165	83
XIV	Synthesis of Pyrazino[2',3',:4,5]thieno[3,2-d]-pyrimidin-2,4-(1H,3H)-dione	85
XV	Synthesis of Thieno[2,3-b]pyrazine	86

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF FIGURES

Number		Page
	Infrared Spectra	
1	Reissert Compound of Thieno[3,2-c]-pyridine	142
2	4-(3',4'-Dimethoxybenzyl)thieno[3,2-c]-pyridine	142
3	4-Methylthiophene-2-carboxaldehyde	142
4	3-Methyl-4-oxo-5H-thieno[3,2-c]pyridine	143
5	Ethyl 3-aminothieno[2,3-b]pyridine-2-carboxylate	143
6	Pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4(3H)-one	143
7	4-Chloropyrido[3',2':4,5]thieno[3,2-d]-pyrimidine	144
8	4-Hydrazinopyrido[3',2':4,5]thieno[3,2-d]-pyrimidine	144
9	Pyrido[3',2':4,5]thieno[3,2-d]pyrimidine	144
10	*s*-Triazolo[4,3-c]pyrido[3',2':4,5]thieno-[3,2-d]pyrimidine	145
11	Tetrazolo[1,5-c]pyrido[3',2':4,5]thieno-[3,2-d]pyrimidine	145
12	3-Methylpyrido[3',2':4,5]thieno[3,2-d]-pyrimidine	145
13	α-Mercaptoacetamide	146

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF FIGURES (Continued)

Number		Page
14	3-Aminothieno[2,3-b]pyridine-2-carboxamide	146
15	3-Aminothieno[2,3-b]pyridine-2-carbonitrile	146
16	2,4-Diaminopyrido[3',2':4,5]thieno[3,2-d]-pyrimidine	147
17	3-Amino-2-acetylthieno[2,3-b]pyridine	147
18	2-Benzylmercaptonicotinonitrile	147
19	Ethyl 3-Hydroxythieno[2,3-b]pyridine-2-carboxylate	148
20	3-Hydroxythieno[2,3-b]pyridine	148
21	Ethyl 7-Aminothieno[2,3-b]pyrazine-6-carboxylate	148
22	Ethyl 7-Amino-2,3-dimethylthieno[2,3-b]-pyrazine-6-carboxylate	149
23	Pyrazino[2',3':4,5]thieno[3,2-d]pyrimidin-4(3H)-one	149
24	4-Chloropyrazino[2',3':4,5]thieno[3,2-d]-pyrimidine	149
25	4-Hydrazinopyrazino[2',3':4,5]thieno[3,2-d]-pyrimidine	150
26	*s*-Triazolo[4,3-c]pyrazino[2',3':4,5]thieno-[3,2-d]pyrimidine	150
27	7-Aminothieno[2,3-b]pyrazine-6-carboxamide	150
28	Pyrazino[2',3':4,5]thieno[3,2-d]-*v*-triazine-4(3H)-one	151

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF FIGURES (Continued)

Number		Page
29	7-Aminothieno[2,3-b]pyrazine-6-carboxamide	151
30	7-Aminothieno[2,3-b]pyrazine-6-carboxylic acid	151
31	2-Methyl-4H-pyrazino[2',3':4,5]thieno-[3,2-d][3,1]oxazin-4-one	152
32	2-Methylpyrazino[2',3':4,5]thieno[3,2-d]-pyrimidin-4(3H)-one	152
33	Pyrazino[2',3':4,5]thieno[3,2-d]pyrimidin-2,4(1H,3H)-dione	152
34	7-Aminothieno[2,3-b]pyrazine	153
35	Ethyl 7-Chlorothieno[2,3-b]pyrazine-6-carboxylate	153
36	Ethyl Thieno[2,3-b]pyrazine-6-carboxylate	153
37	Thieno[2,3-b]pyrazine	154
	PMR Spectra	
38	3-Methyl-4-oxo-5H-thieno[3,2-c]pyridine	155
39	3-Methyl-4-chlorothieno[3,2-c]pyridine	155
40	3-Methylthieno[3,2-c]pyridine	155
41	4-(3',4'-Dimethoxybenzyl)thieno[3,2-c]-pyridine	156
42	Compound 113	156
43	7-Aminothieno[2,3-b]pyrazine-6-carboxamide	156
44	Ethyl 7-aminothieno[2,3-b]pyrazine-6-carboxylate	157

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF FIGURES (Continued)

Number		Page
45	Ethyl 7-Chlorothieno[2,3-b]pyrazine-6-carboxylate	157
46	Ethyl Thieno[2,3-b]pyrazine-6-carboxylate	157
47	Thieno[2,3-b]pyrazine-6-carboxylic acid	158
48	7-Aminothieno[2,3-b]pyrazine	158
49	Thieno[2,3-b]pyrazine	158
50	Pyrazino[2',3':4,5]thieno[3,2-d]pyrimidine	159
51	Ethyl 3-Hydroxythieno[2,3-b]pyridine-2-carboxylate	159

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

STUDIES INVOLVING THIENOPYRIDINE AND THIENOPYRAZINE ANALOGS OF BIOLOGICALLY ACTIVE MOLECULES

by

FRED WAYNE CLOUGH

An Abstract

Of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry of the University of South Florida

March, 1976

Thesis Supervisor: Dr. Stewart W. Schneller
Associate Professor

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ABSTRACT

Attempts to prepare 3-(β-aminoethyl)thieno[3,2-c]-pyridine from 3-methylthieno[3,2-c]pyridine are presented but were unsuccessful. The halogenation of thieno[3,2-c]-pyridine was also unsuccessful and a rationalization for this is presented. However, application of the Reissert reaction on thieno[3,2-c]pyridine, followed by subsequent elaboration, did produce an alkaloid analog of papaverine in which a thiophene ring replaces a benzene ring of papaverine.

Pyrido[3',2':4,5]thieno[3,2-d]pyrimidine and a number of its derivatives have been prepared. Included among these derivatives is 2,4-diaminopyrido[3',2':4,5]thieno[3,2-d]-pyrimidine, a compound containing the essential chemical features common to many antimalarial agents. Simple syntheses of conveniently substituted thieno[2,3-b]pyridines are also presented.

The synthesis of pyrazino[2',3':4,5]thieno[3,2-d]-pyrimidine and various 4-substituted derivatives was accomplished. These include pyrazino[2',3':4,5]thieno[3,2-d]-pyrimidin-2,4-(1H,3H)-dione, a key intermediate in the future systhesis of separated folic acid analogs. Also, the previously unreported thieno[2,3-b]pyrazine has been prepared along with many potentially useful intermediates. These include 7-chlorothieno[2,3-b]pyrazine and ethyl thieno[2,3-b]-pyrazine-6-carboxylate which complement each other in that

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the former exhibits the potential for functionalization at the C-7 position, whereas, the latter would allow functionalization specifically at the C-6 position.

Finally, the molecular orbital data for thieno[2,3-b]-pyrazine and thieno[3,4-b]pyrazine are presented and analyzed in detail.

Abstract approved: Stewart W. Schneller, thesis supervisor
Associate Professor, Department of Chemistry
November 15, 1975

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

PREFACE

The material contained in this thesis represents the preliminary work completed on a number of continuing projects. The background material, although accumulated under two headings (Biological and Chemical), is presented in sections corresponding to the various projects discussed. The background material is extensive, but justifiably so since it is intended to precisely establish the rationale and governing principles for each project.

The material presented herein deals with only the synthetic endeavors directed toward various heterocyclic systems. For instance, the synthesis of 3-(β-ethylamine)-thieno[3,2-c]pyridine was attempted. This compound is one of three compounds that are necessary for studies directed at more precisely defining the structure-activity relationships of the biologically important molecule serotonin. Secondly, attention is focused, in this thesis, on the synthesis of 2,4-diaminopyrido[3',2':4,5]thieno[3,2-d]pyrimidine as a potentially useful antimalarial agent. Finally, the synthesis of the molecular framework (*i.e.*, pyrazino[2',3':4,5]thieno-[3,2-d]pyrimidine) of one of a number of separated folic acid analogs was undertaken. Utilization of the synthetic procedures elucidated here will make the synthesis of the actual separated folic acid analogs a reality, thus, establishing

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

more definately the geometrical relationships between the functionalities of folic acid and methotrexate that are necessary to impart biological activity.

These were the main objectives and, as a consequent of pursuing them, a great deal of the chemistry of the fundamental ring systems resulted. The presentation of these results is an integral part of this thesis.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

"Einem ist sie die hohe, die himmlische
Gottin, dem anderen eine tuchtige Kuh,
die ihn mit Butter versorgt."

- F. Schiller
Die Wissenschaft, 1796

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

INTRODUCTION

The discovery and introduction into medicine of chemicals not previously known to have useful medicinal properties is the domain of the medicinal chemist. Although he is necessarily a member of a research team comprised of chemists, biochemists, pharmacologists and physicians working in close cooperation with one another, the medicinal chemist is called upon to bridge the interdisciplinary prejudices and formulate a composite analysis of the situation beyond that which a specialist in any one field could achieve. Unfortunately, the medicinal chemist faces many obstacles-- not the least of which is the lack of a simple correlation between chemical structure and biological activity-- in the design of new drugs possessing specific biological activity. The development of tables[1] which relate functional groups to biological activity has attempted to alleviate this difficulty. However, these are admittedly of limited usefulness because they have often been formulated without precise knowledge of the manner in which drugs intercede in delicately balanced biological processes.

On occasion, the medicinal chemist is aided by the discovery of natural products possessing exceptional medicinal value. Perhaps two of the more important of these in the last thirty years have been the discovery[2] that the

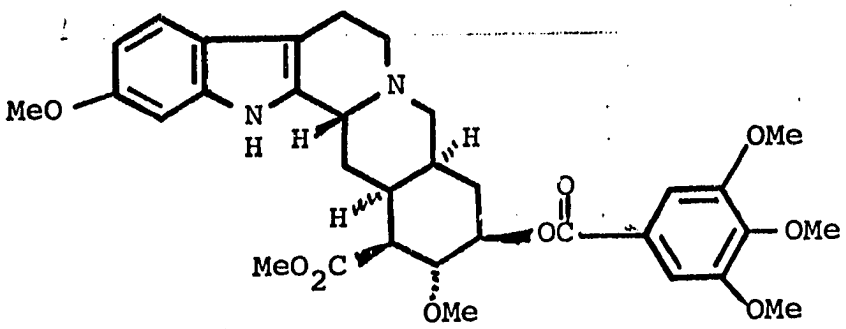
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Rauwolfia alkaloid reserpine (1; 11,17α-dimethoxy-16β-carbomethoxy-18β-(3,4,5-trimethoxybenzoyloxy)-3β,20α-yohimbine) was an effective antihypertensive agent and that the periwinkle (*Vinca rosea*. Linn.) alkaloids vinblastine (2) and vincristine (3) were effective anticancer agents.[3] These are, however, exceptions and not generally the rule for biologically active compounds derived from botanical sources and ordinarily a medicinal chemist must depend on his ingenuity to design effective biologically active agents.

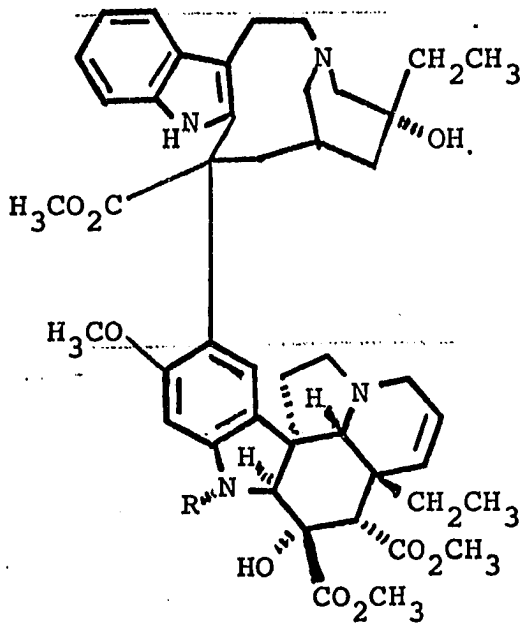
In view of the complex nature of the biochemistry of an organism, it is not surprising that compounds foreign to such a system (*i.e.*, drugs) possess inherent problems, the manifestations of which are often undesirable side reactions. In order to surmount these obstacles, the medicinal chemist often begins with a compound of known biological activity and employs the process of molecular modification with the intention of improving its biological specificity and response while maintaining or decreasing its inherent side effects.

The work described in this thesis draws upon both the concepts of molecular modification and bioisosterism in attempts to rationally produce compounds which either (i) aid in more rigorously defining the molecular parameters necessary for the activity of known biologically active compounds, or (ii) are of potentially greater therapeutic

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



1

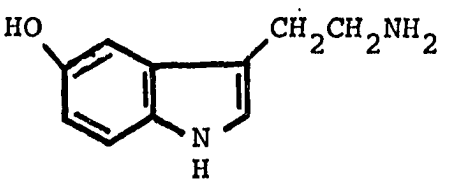


2; R= CH_3

3; R= CHO

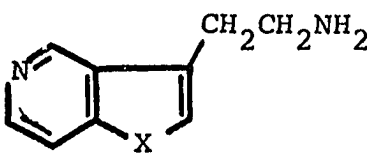
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

value than the corresponding parent agent. For example, serotonin (4) is a biologically active molecule which is a potent stimulator of smooth muscle tissue and implicated as a possible neurohormone. In its capacity as a neurohormone,



4

there exists little conclusive data on the relative bio-importance of its 5-hydroxyl substituent nor on the degree of interdependence of the 5-hydroxyl substituent and the indole ring nitrogen in the binding of 4 to the bio-receptor molecule. Thus, application of (i) the concept of molecular modification by substituting a ring nitrogen for the 5-hydroxyl substituent and then (ii) the concept of bioisosterism by replacing the indole ring nitrogen with either divalent sulfur or oxygen would result in a series of compounds (5,6,7). A comparison of the pharmacological

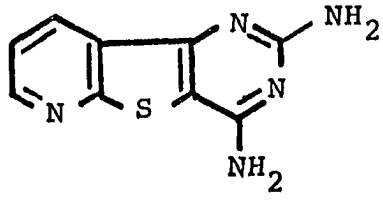


5; X= NH
6; X= O
7; X= S

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

screening results acquired from these three compounds would provide insight into the answers to both of these questions regarding substituent bio-involvement.

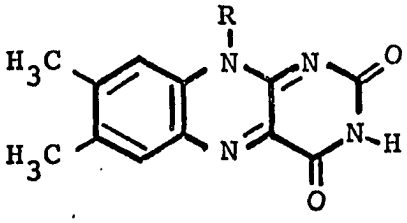
In the field of antifolates as antimalarial agents, the 2,4-diaminopyrimidine moiety is a frequent molecular feature in many of the most active compounds. There exists no examples of this moiety fused to the completely aromatic thienopyridine nucleus. Thus, the synthesis of compound 8 and various derivatives of the parent tricyclic

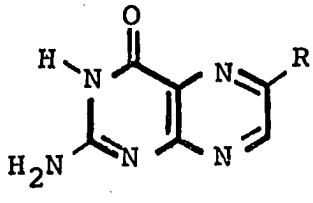


8

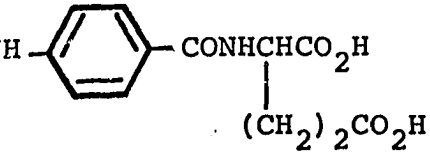
system has been pursued. This study also produced valuable groundwork chemistry for a variety of projects described in this thesis.

Finally, riboflavin (9), an important cofactor in biological oxidation-reduction reactions, and folic acid (10),



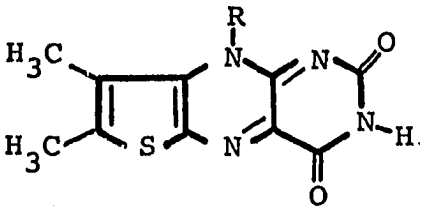


9; R= $CH_2(CHOH)_3CH_2OH$

10; R= 

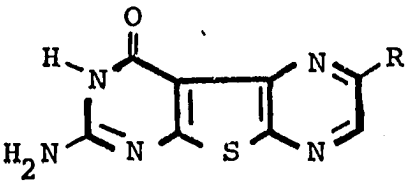
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

whose 7,8-dihydro and 5,6,7,8-tetrahydro forms are important coenzymes in cellular growth are two other biologically important molecules which have been of interest. In previous studies on riboflavin, very little attention has been devoted to the bio-significance of the benzene ring. Therefore, it became meaningful here to synthesize analogs of riboflavin in which the benzene ring was replaced by an isosteric thiophene ring (11).



11; R= $CH_2(CHOH)_3CH_2OH$

In the case of folic acid the question arose as to the biological importance of (i) fusion of the pyrazine and pyrimidine rings and (ii) the relationships between the various functional groups of 10. Therefore, an investigation was undertaken into the synthesis of a series of compounds in which these rings were separated by an atomic bridge (*eg.*, 12).



12; R= CH_2NH–C$_6$H$_4$–$CONHCH(CO_2H)(CH_2)_2CO_2H$

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

An essential molecular feature of both the riboflavin and folic acid studies was the presence of the thieno-pyrazine moiety (13). Because of the lack of reports on the synthesis of this ring system, a great deal of work presented herein deals with the synthesis and various conversions of this molecule.

13

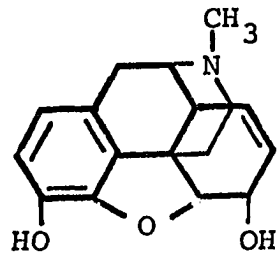
Before continuing and in order to facilitate further discussions, it is appropriate at this juncture to define explicitly what is involved in the concepts of molecular modification and bioisosterism. Therefore, these terms are discussed at length below.

Molecular Modification

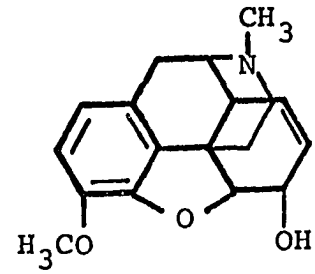
Molecular modification[4] is the systematic variation of the structure of a metabolite or synthetic compound of known biological activity and the subsequent evaluation of the pharmacodynamic properties of the resulting molecule. This process is most effective when the modification introduced is at first very simple since the effect of this molecular change on the biological response can be easily monitored and readily reconciled. This rationale arises

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

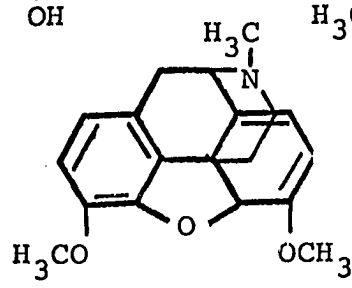
from the fact that apparent minor alterations in chemical structure can produced drastic changes in biological activity. This subtle effect can be illustrated with opium which is a complex mixture of at least twenty-three alkaloids. However, the three major constituents are morphine (14), codeine (15) and thebaine (16), which all contain the morphinan ring system. Morphine (14) is a



14



15



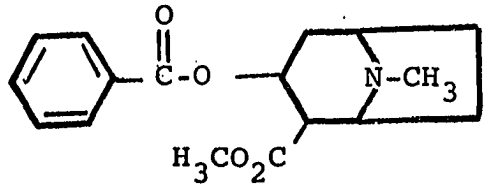
16

potent analgesic yet conversion of its phenolic group to a methoxyl group results in a molecule (*ie.*, codeine (15)) possessing only one-tenth of the analgetic activity of 14, and still has useful antitussive activity. Furthermore, methylation of the allylic hydroxyl group of 15 and removal of two hydrogens results in thebaine (16) which is neither an analgetic (as 14) nor an antitussive (as 15), but is a rather potent poison resembling strychnine and brucine in its activity.[5]

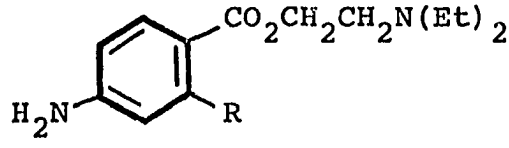
Nonetheless, the process of molecular modification has

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

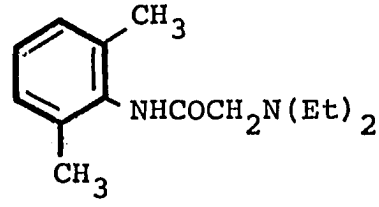
been extremely useful and successful in a number of cases. For example, naturally occuring cocaine (17) possesses topical anesthetic activity,[6] but is of little value as a local anesthetic.[4] However, variation in the structure of cocaine led to the synthesis of procaine (18), chloroprocaine (19), and eventually to the potent local anesthetic lidocaine (20).



17



18; R= H
19; R= Cl

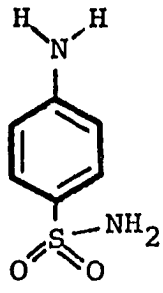


20

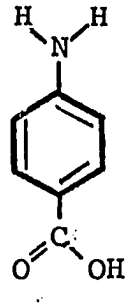
Molecular modification has also assisted in the understanding of the relationship between a drug and its bio-receptor. Such studies have evolved into a receptor theory of drug action[7] which stresses the importance of geometry and electronic distribution in the drug molecule as a determining factor in its biological activity. No drug receptor has yet been isolated or identified and therefore its physical nature necessarily remains obscure. However, it has long been recognized that many substances which inhibit an enzyme competitively often-times are structurally analogous to the enzyme's natural

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

substrate. Thus, an advantageous starting point for the synthesis of potential biologically active compounds would be the creation of substances chemically related to a natural metabolite which may then interfere with the normal function of that metabolite in living cells. Such substances are referred to as antimetabolites. A classical example is sulfanilamide (21) which is structurally similar to p-aminobenzoic acid (22), an essential element in the growth



21



22

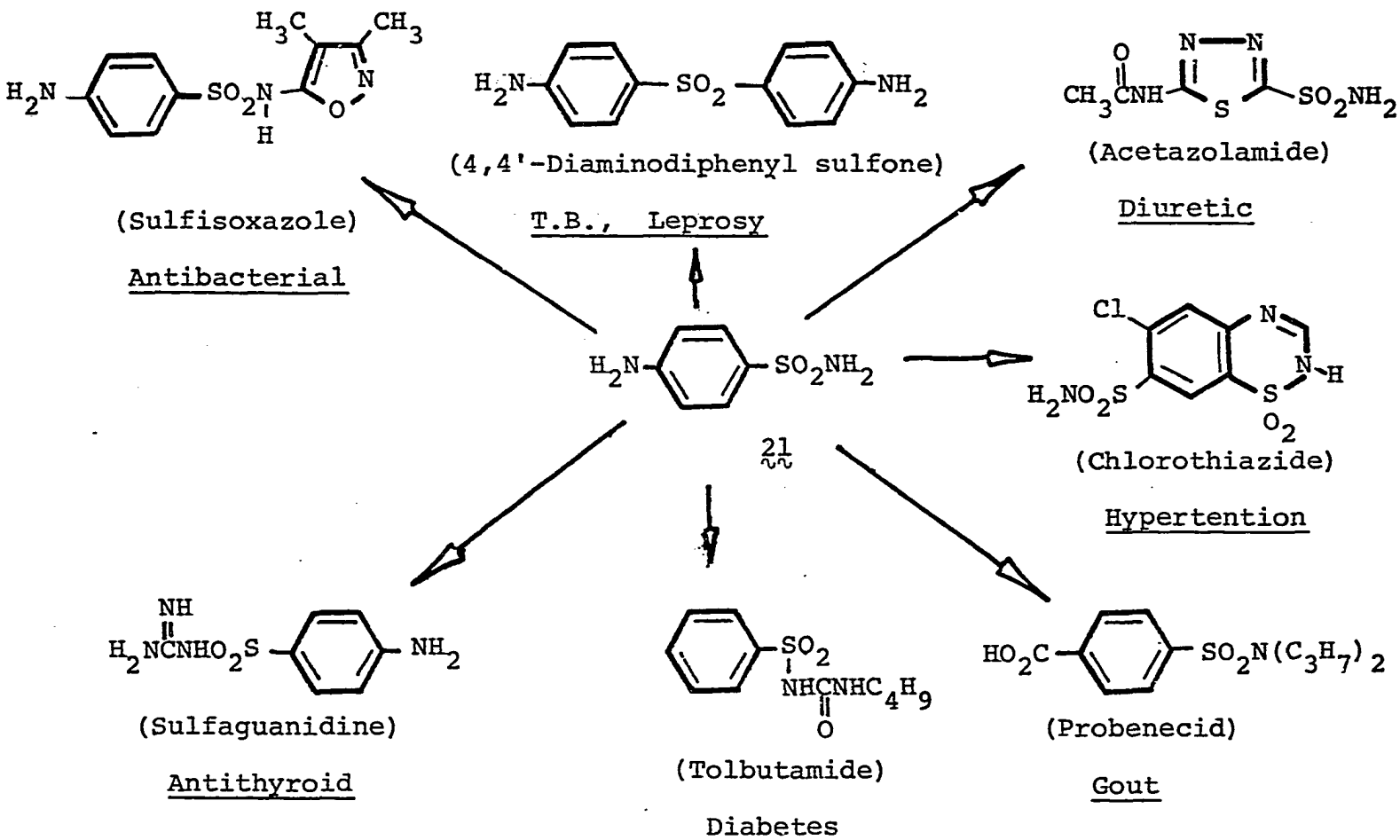
of many bacteria.[8,9] In this instance, sulfanilamide (21) interferes with the utilization of 22 in the synthesis of dihydropteroic acid by micro-organisms. In contrast, mammalian cells are not affected by this agent since they are unable to synthesize dihydropteroic acid *de novo* and require it preformed. Thus, compound 21 acts as a selective antimetabolite of 22 by preventing its utilization in dihydropteroic acid synthesis by micro-organisms with no effect on mammalian cells.[10] From this investigation, medicinal chemists have since molecularly modified the structure of 21 to produce a diverse number of therapeutically useful compounds (Chart 1) which are currently being utilized in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

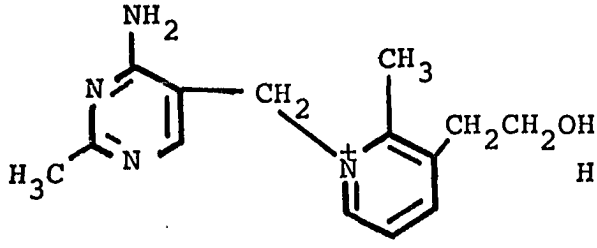
Chart I

Molecular Modification of the Sulfanilamide Structure

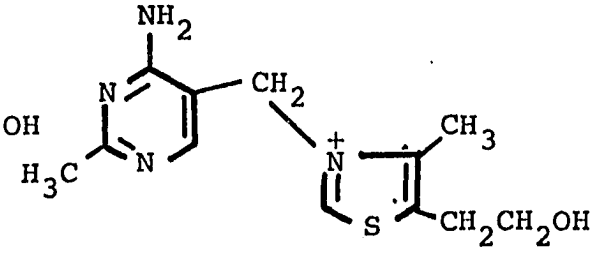


the treatment of bacterial infections, leprosy. hypertension, gout, antithyroidism, and diabetes.[5]

Antimetabolites can often be designed by suitable molecular modification of a natural metabolite via substitution, homologation and/or isomerization. For example, pyrithiamine (23) is an antimetabolite of the essential vitamin thiamine (24). The only difference between these two compounds is that a pyridine ring in 23 has replaced



23



24

the thiazole ring in 24. This approach to molecular modification in the production of effective agents is an example of the application of the concept of bioisosterism.

Bioisosterism

The concept of isosteric replacement aids the medicinal chemist in the search for more specific and more effective biological agents. In this approach, an atom or group of atoms in the lead compound are replaced by other atoms which possess similar electronic and steric configurations. This

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

concept was first introduced by Langmuir[11] and evolved from his consideration of the consistencies observed in the properties of the elements in the periodic chart. He concluded that two molecules or ions possessing identical numbers and arrangements of electrons and atoms should exhibit similar properties. Examples of Langmuir's isosteres are CO and N_2, CO_2 and N_2O, and N_3^- and NCO^-.

In an extension of this, Hinsberg[12], acknowledging the close similarities in the chemical and physical properties of benzene, thiophene, and pyridine, formulated the concept of "ring equivalents". By his postulate, the vinylene (-CH=CH-) group and divalent sulfur (-S-) and the trivalent nitrogen (-N=) and methylenic carbon (-CH=) are considered ring equivalents. The concept of isosterism and ring equivalency was elaborated in 1925 by Grimm[13] when he published his "hydride displacement law" which stated that "atoms anywhere up to four places in the periodic chart before an inert gas change their properties by uniting with one to four hydrogens, in such a manner that the resulting combinations behave like pseudoatoms (Grimm's term for Langmuir's isosteric molecules), which are similar to elements in the group one to four places, respectively to their right." These relationships are summarized in Table I.

Erlenmeyer[14] accepted Grimm's classification and broadened the definition of isosteres to "atoms, ions, or molecules in which the peripheral layer of electrons can be considered to be identical." The modern medicinal

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

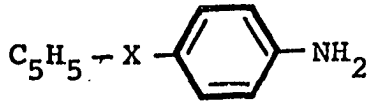
Table 1

Grimm's Hydride Displacement Law

Number of Electrons					
6	7	8	9	10	11
=C=	-N=	-O-	F-	Ne	Na^+
	-CH=	-NH-	OH-	FH	
		$-CH_2-$	NH_2-	OH_2	
			CH_3-	NH_3	OH_3^+
				CH_4	NH_4^+

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

chemist's interest in the relationship of biological activity to the concept of isosterism stems from Erlenmeyer's[15] initial demonstration that the isosteres 25 all exhibit antigen activity. Realizing the potential value of the concept of isosterism in assisting in the solution to



25; X= NH, CH_2, O

biological problems, Friedman[16] suggested that the term "bioisosterism" be applied to compounds which "fit the broadest definition of isosteres and have the same type of biological activity."

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

BIOLOGICAL BACKGROUND

I. Serotonin

Almost a century ago, the vasocontrictor properties of a serum from clotted blood were noted. In the late 1940's this substance was explored as a humoral pressor agent which might be involved in arterial hypertension. Then in 1949, these investigators isolated the vasoconstrictor substance as a complex[17] which they named serotonin and deduced its structure[18,19] to be 5-hydroxytryptamine (4). Coincidentally, at about the same time, Von Erspamer and Asero[20] were studying a substance they called enteramine and which imparted peculiar histochemical properties to the enterochromaffin cells of the mammalian gastrointestinal mucosa. They soon determined that this substance was identical to the active principle isolated from clotted blood, and, therefore, was also serotonin. Since this auspicious beginning, an enormous interest in this amine and related indolealkylamines has resulted[21,22] and evolved into a research pursuit which has received much attention.[23]

Serotonin (4) is widely distributed in nature, being found in mammals, birds, fish, and in fruits such as pineapples, bananas, plums, and nuts. It is also present in numerous venoms including those of the wasp and scorpion.[24] In man, of the total amount of 4 present (5-10 mg), 90% is found in the intestine and the remaining 10% in blood platelets and the brain.[24] In tissues other than blood

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

platelets, 4 is locally synthesized by the required enzyme systems, while blood platelets lack these required enzymes and must acquire the free amine from the blood.

The major biosynthetic pathway for serotonin[25] begins with tryptophan (28, Chart II) which is hydroxylated to 5-hydroxytryptophan (5-HPT, 29) by the enzyme tryptophan-5-hydroxylase. Subsequent decarboxylation of 5-HPT by the enzyme 5-hydroxytryptophan decarboxylase results in 4. The alternative pathway which proceeds by decarboxylation of 28 to give tryptamine (26, X=R=R'=H), followed by hydroxylation has been shown to result in 6-hydroxytryptamine and not 4.[26]

Serotonin is metabolized by monoamine oxidase, converting it to 5-hydroxyindoleacetaldehyde, which is then enzymatically oxidized further by the action of an aldehyde dehydrogenase. There are also a number of minor metabolic pathways involving oxidation, conjugation, O-methylation, N-acetylation and transamination.[21]

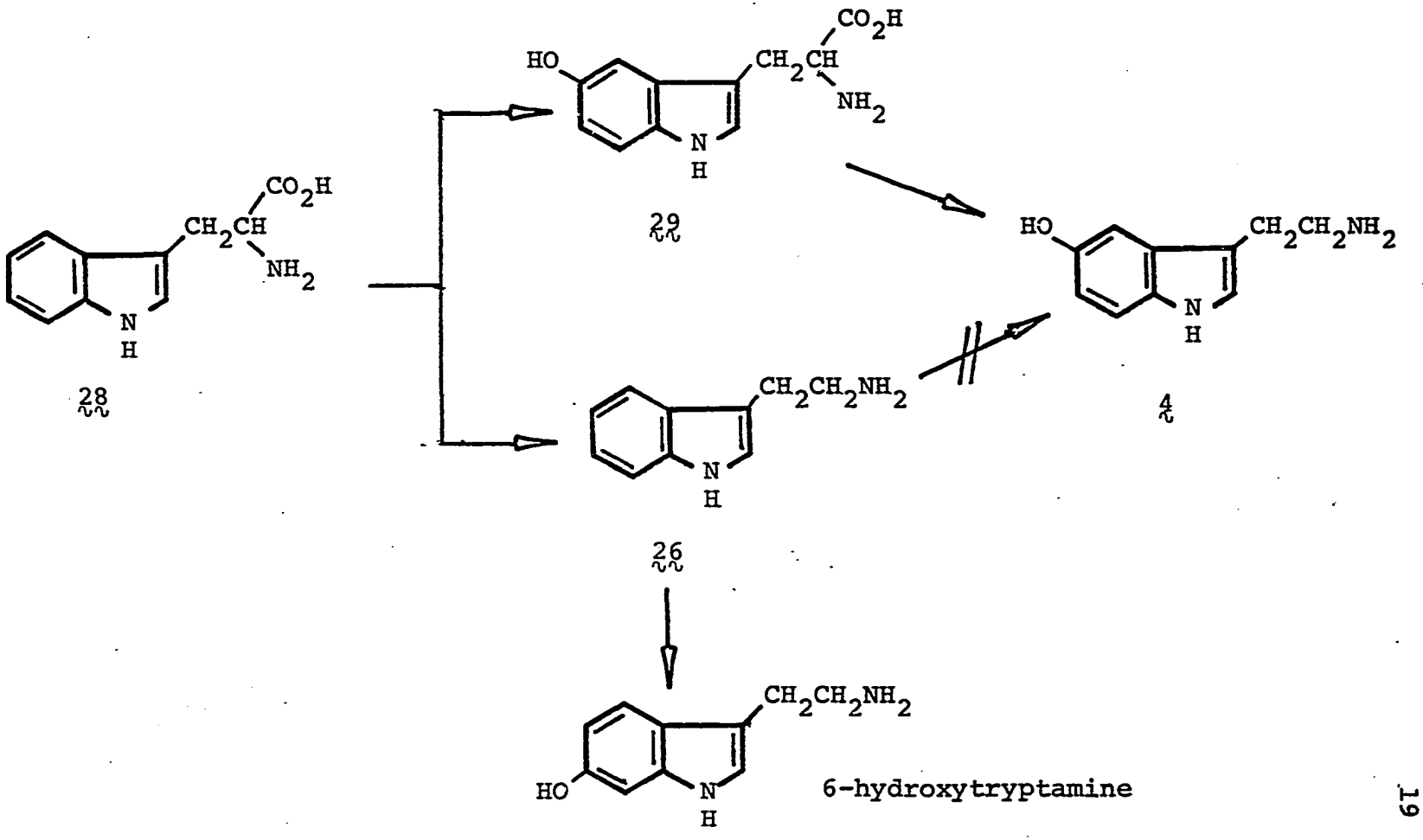
The biological activity of serotonin is diverse, involving the stimulation of a variety of smooth muscles and nerves, producing a broad spectrum of responses involving the respiratory, gastrointestinal, cardiovascular, and central nervous systems. Of particular interest here is the interaction of serotonin with the central nervous system. Serotonin is present[27] although unevenly distributed in the mammalian brain,[28] being found in relatively high concentrations in the cortex and cerebellum. The distribution of 5-HPT decarboxylase in the brain has been shown[29] to correlate well

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chart II

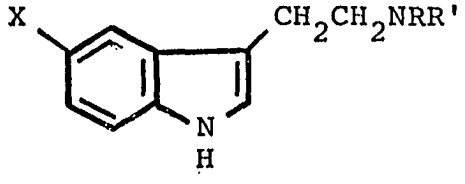
Biosynthesis of Serotonin



with that of serotonin. This is not surprising since serotonin is unable to cross the blood-brain barrier whereas its biosynthetic precursor, 5-HPT, possesses this capability.

The actual function of serotonin in the brain is yet to be unequivocally determined. However, several facts suggest that serotonin may be acting as a neurohormone within the central nervous system. First, the enzymes for both the synthesis and degradation are present within the brain. Secondly, the turnover of the substance is very rapid, and finally, the uneven distribution and storage all suggest a specific function in neural activity.[30]

The wide ranging physiological and pharmacological effects of 5-substituted indolealkylamines (26) are not completely understood. Indeed, the diversified effects of structural variation on biological activity of indolealkylamines has received much attention.[31] This is indicated



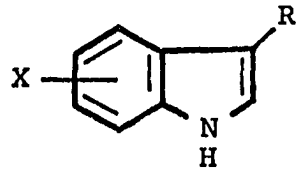
26

by a summary of the studies of Vane[32] in Table 2. Basing his assumption on the fact that 5-hydroxytryptamine (serotonin, 4) and its non-phenolic analogs (26, X=H) act at the same receptor sites, the following observations can be made:

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 2

Relative Activities of Some Tryptamine and 5-Hydroxy-tryptamine Derivatives[32]



No.	X	R	relative molar activities* before	after
1	5-OH	$CH_2CH_2NH_2$	1.0	1.0
2	H	$CH_2CH_2NH_2$	.408	52
3	H	CH_2NH_2	29000	33000
4	H	$CH_2CH_2CH_2NH_2$	1920	460
5	H	$CH_2CH_2NHCH_3$	1120	96
6	H	$CH_2CH_2NHC_2H_5$	250	170
7	H	CH_2CH_2NH-n-C_3H_7	230	330
8	H	$CH_2CH_2N(CH_3)_2$	196	110
9	H	$CH_2CH_2N(C_2H_5)_2$	83	87
10	H	CH_2CH_2N-(n-$C_3H_7)_2$	34	41
11	4-OH	$CH_2CH_2NH_2$	1.8	2.0
12	6-OH	$CH_2CH_2NH_2$	460	560
13	5-OCH_3	$CH_2CH_2NH_2$	20	1.7
14	6-OCH_3	$CH_2CH_2NH_2$	1520	950
15	5,6-$(OCH_3)_2$	$CH_2CH_2NH_2$	300	147
16	5-Cl	$CH_2CH_2NH_2$	100	7.6
17	5-CH_3	$CH_2CH_2NH_2$	184	9
18	5-OH	$CH_2CH_2NHCH_3$	6.3	9.5
19	5-OH	$CH_2CH_2N(C_2H_5)_2$	20	20
20	5-OH	CH_2CH_2N-(n-$C_3H_7)_2$	4	6

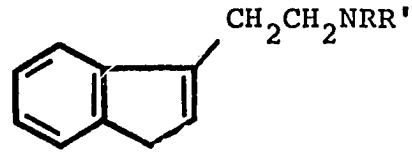
* Before and after MAO inhibition.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1) methylamine and propylamine side chains are 1000 and 15 times less active, respectively, than the ethylamine side chain,
2) the activity is more or less maintained with the isomeric 4-hydroxytryptamine but drastically reduced with 6-hydroxy-tryptamine, and
3) 5-methoxy-, 5-methyl-, 5-chloro-, and 5-hydroxytryptamines are all more active than tryptamine itself.

The conclusions which can be drawn suggest that the 3-ethylamine side chain represents an optimal structural feature while the nature of the 5-substituent is less critical for biological activity. On the other hand, it has been suggested[32] that the 5-hydroyl and the 3-terminal amino groups may be involved in the binding of serotonin (4) to its receptor. Thus, from a correlation of these studies it is apparent that the biological role of the 5-substituent is not well understood.

The studies of Gessner and co-workers[33] have considered the importance of other sites, particularly the indole ring nitrogen, on the biological activity of derivatives of tryptamine (26, X=R=R'=H) and serotonin (4). Their data is presented in Table 3 and was obtained from comparing the activities of substituted tryptamines and their indene isosteres (27). This data suggests that the indole ring

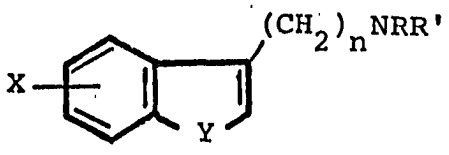


27

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 3

Relative Affinities and Intrinsic Activities of Substituted Tryptamines and Their Indene Isosteres[33]



No.	X	Y	n	R	R'	Relative intrinsic Affinity (activity)
1	5-OH	NH	2	H	H	1.00
2	H	NH	2	H	H	1.32
3	H	NH	3	H	H	0.72
4	H	CH_2	3	H	H	0.49
5	H	NH	2	CH_3	CH_3	1.32
6	H	CH_2	2	CH_3	CH_3	1.27
7	*H*	*NH*	*2*	C_2H_5	C_2H_5	*1.01*
8	H	CH_2	2	C_2H_5	C_2H_5	1.39
9	H	NH	2	$CH(CH_3)_2$	i-Pr	1.51
10	H	CH_2	2	i-Pr	-Pr	0.91
11	5-OCH_3	NH	2	C_2H_5	C_2H_5	1.98
12	*6-OCH_3*	*NH*	*2*	C_2H_5	C_2H_5	*0.19*
13	*6-OCH_3*	*CH_2*	*2*	C_2H_5	C_2H_5	*1.11*

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

nitrogen of 26 (X=H) does not play a role in the binding of tryptamines to the receptor molecule.

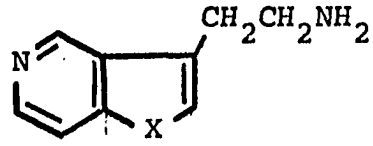
However, it is pertinent to note that although 5-methyl-N,N-diethyltryptamine (Table 2, entry 17) is very active, 6-methoxy-N,N-diethyltryptamine (Table 3, entry 12) is virtually inactive. In contrast, 3-(2-(N,N-diethylamino-ethyl))-6-methoxyindene (Table 3, entry 13) is quite active. This suggests and corroborates the previously expressed belief[34,35,36] that tryptamines and 5-hydroxytryptamines may be acting at different receptor sites.

Therefore, it is apparent that there are still a number of questions that remain unanswered regarding (i) the specific roles of the 5-substituent and the indole ring nitrogen and (ii) the degree of involvement and interdependency of the 5-hydroxyl substituent and the ring nitrogen in determining the biological activity of serotonin (4) and related molecules.

It was the intention here to explore the role and degree of involvement and interdependency of the 5-hydroxyl group and the ring nitrogen of 4 by replacing the 5-hydroxyl substituent of 4 with a sterically different but electronically similar ring nitrogen. Subsequently, the indole ring nitrogen would be replaced by bioisosteric oxygen and sulfur atoms. This substitution sequence would produce three analogs of serotonin, specifically, 3-(β-aminoethyl)-5-azaindole (5), 3-(β-aminoethyl)furo[3,2-c]pyridine (6), and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3-(β-aminoethyl)thieno[3,2-c]pyridine (7).



5; X= NH

6; X= O

7; X= S

By correlating the biological screening data of 5 with that of the appropriate 26, it will be possible to further assess the importance and involvement of the 5-hydroxyl substituent in the biological activity of 4. Furthermore, a comparison of the pharmacological activities of 6 and 7 will help to define more rigorously the role of the indole ring nitrogen of 4 in its biological activity. Finally, when this complete set of data is integrated, the question of a two point interaction or interdependency between the indole ring nitrogen and the 5-substituent upon the biological receptor should begin to be more precisely defined.

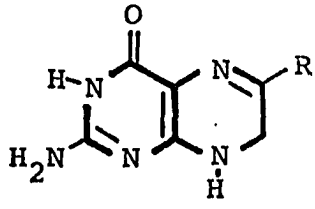
This thesis describes the synthetic work completed in pursuit of compound 7, whereas the research involved in the preparation of 5 and 6 represents a continuing area of exploration. The results of this study will be presented in the Discussion Section of this thesis.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

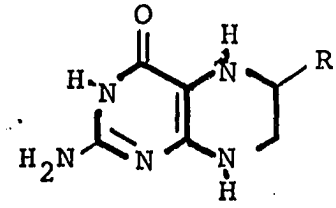
II. Chemotherapeutic Antifolates

A great deal of attention has been devoted to chemotherapeutic agents (for example, antimalarials and antineoplastics) based on folate antagonism. Interest in this field began in the 1940's with the early studies on the structure elucidation and synthesis[37] of the B vitamin folic acid (10). Considerable interest then evolved which focused on understanding the vital role of the ubiquitous folic acid and its coenzymes in cellular metabolism.[38,39] This in turn stimulated an overwhelming interest in the application of folate antagonists as medicinal agents.[40-43]

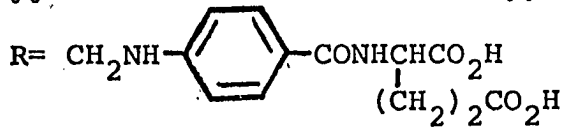
Such a chemotherapeutic approach depends on the fact that mammals require exogenous folic acid as an essential growth factor which they reduce enzymatically to the biocritical cofactor forms, 7,8-dihydrofolic acid (30) and 5,6,7,8-tetrahydrofolic acid (31). In contrast, many



30



31



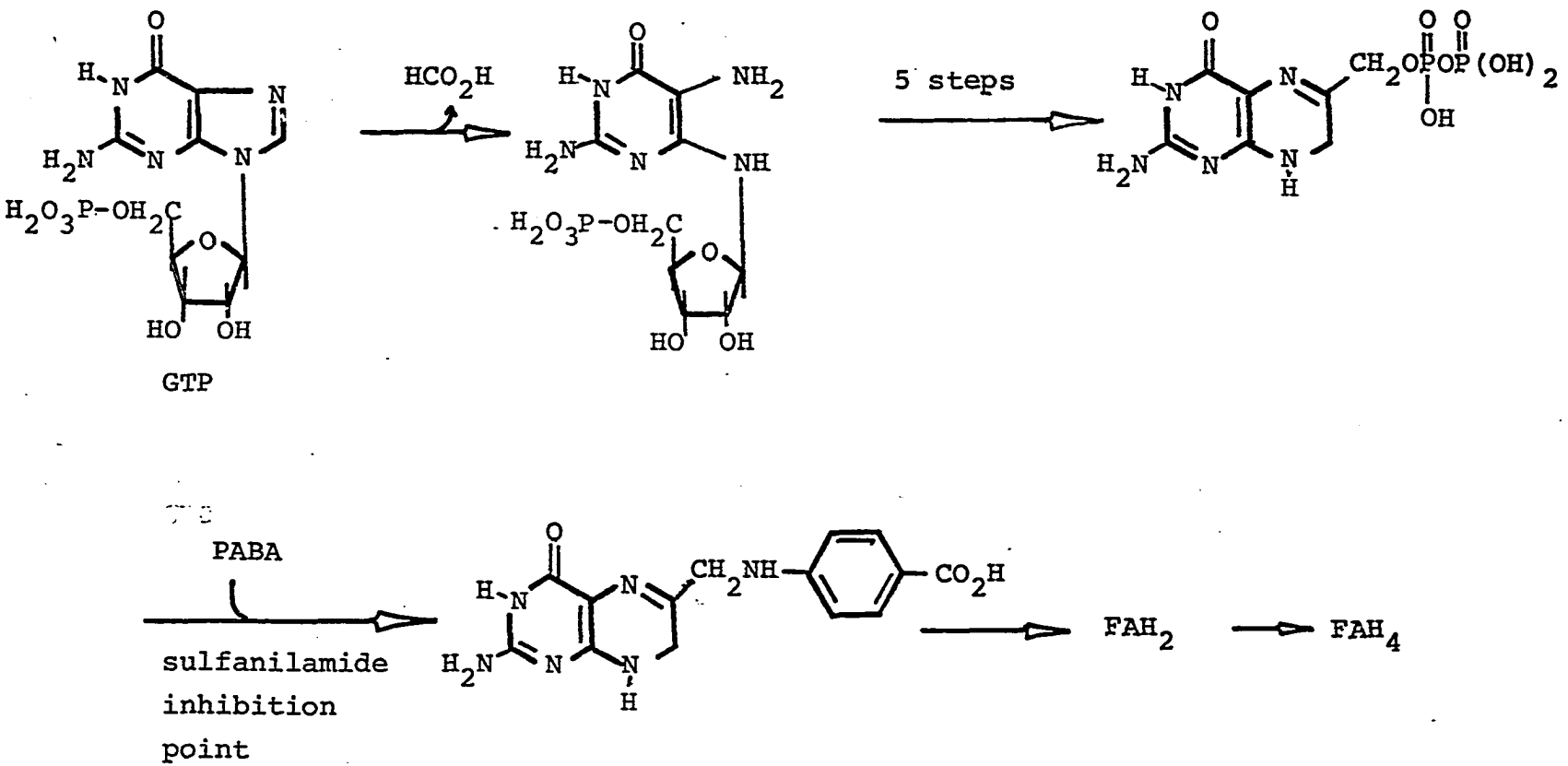
parasites and bacteria are unable to utilize the preformed folates and must synthesize their tetrahydrofolic acid *de novo*[39] from guanosine triphosphate in an eight step synthesis (Chart III).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chart III

de novo Synthesis of Folic Acid From GTP



Tetrahydrofolic acid (31) is vital for normal cellular growth and is utilized in one-carbon enzymatic transfer reactions which include (i) interconversion between several amino acids, (ii) initiation of peptide chain synthesis, (iii) *de novo* synthesis of purine nucleotides and (iv) the *de novo* synthesis of the pyrimidine nucleotide thymidylic acid. Six tetrahydrofolic acid coenzymes have been identified as significant in these processes. These include (Table 4) 5-methyltetrahydrofolic acid (32), 5-formyltetrahydrofolic acid (33), 5,10-methylenetetrahydrofolic acid (34), 5,10-methenyltetrahydrofolic acid (35), 10-formyltetrahydrofolic acid (36), and 5-formiminotetrahydrofolic acid (37) which are particularly important at three vital points in the biosynthesis of nucleic acids (Chart IV):

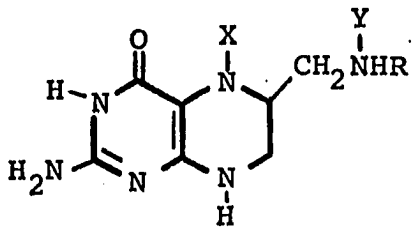
1) molecule 36 supplies the formyl group (ultimately C-2 of the purine ring) necessary for the conversion of 4-amino-5-imidazolecarboxamide ribonucleotide (38) to 4-formamide-5-imidazolecarboxamide ribonucleotide (39),
2) compound 35 donates the formyl group for the conversion of glycinamide ribonucleotide (40) to formylglycinamide ribonucleotide (41) leading ultimately to the insertion of the C-8 carbon of purines, and
3) compound 34 supplies the methyl group necessary for the conversion of 2'-deoxyuridylic acid (42) to thymidylic acid (43).

Exploitation of these essential biochemical pathways has led to the rational design of a number of important chemotherapeutic agents.

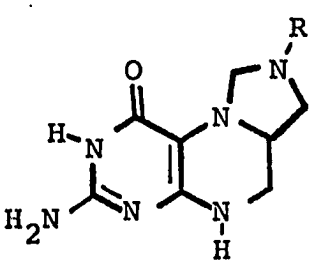
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 4

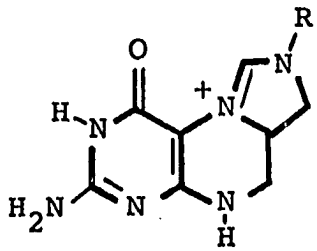
Tetrahydrofolic Acid Coenzymes



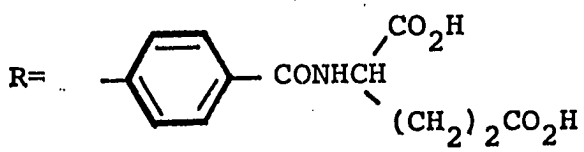
	X	Y
32	CH_3	H
33	CHO	H
36	H	CHO
37	CH=NH	H



34



35

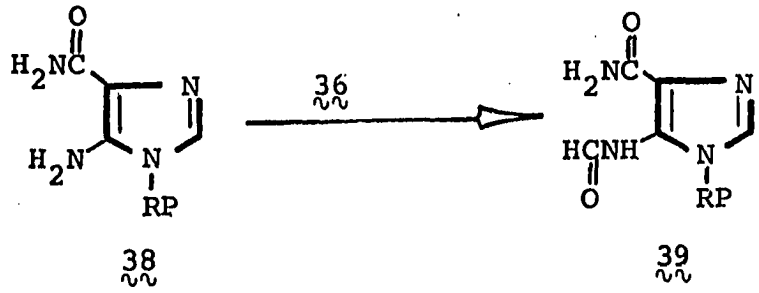


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

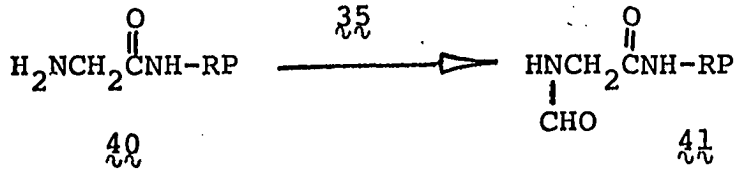
Chart IV

Biological Utilization of

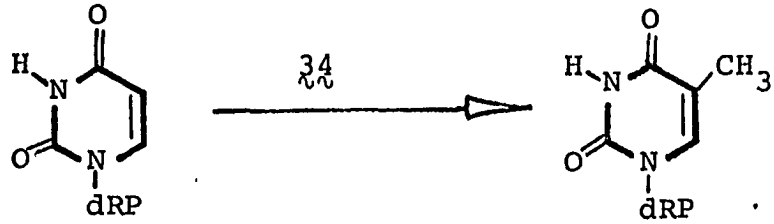
Tetrahydrofolic Acid Coenzymes



38 39



40 41

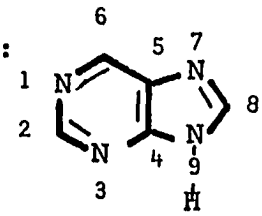


42 43

RP= β-D-ribofuranose phosphate

dRP= 2'-deoxy-β-D-ribofuranose phosphate

Purine Numbering:



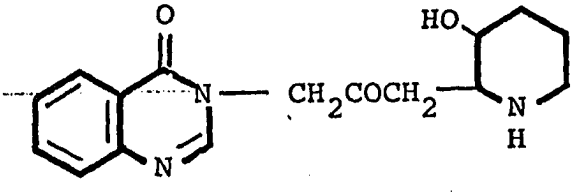
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

A. Antimalarial Agents

> "The clinician has at his disposal a complete series of effective drugs for the treatment of all stages of the disease...."[44]

The disease referred to in the statement above is malaria and in the early 1950's when this optimism was expressed, it was truly believed that this disease had been conquered. Shortly after this statement was made, scattered reports of malarial strains refractory to currently employed drugs[45] began to appear and, therefore, malaria again began its ascent to an infamous position of prominence among the diseases of worldwide concern.

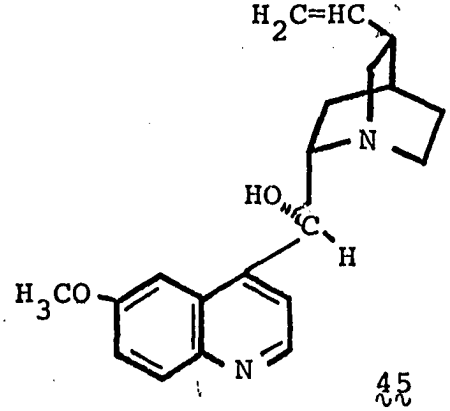
It is interesting to briefly examine the history of the chemotherapeutic approaches to malaria, a disease which has plagued man from his very earliest days. One of the oldest preparations for the treatment of malaria was the Chinese drug "ch'ang shan" which consisted of the powdered roots of *Dichroa febrifuga* and whose active component has been shown[46] to be the alkaloid febrifugin (44).



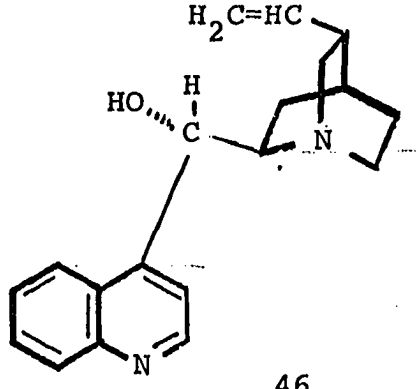
44

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Perhaps the most familiar antimalarial agents, however, are those derived from the cinchona bark which were first recognized as antipyretic agents by the South American Indians as early as the beginning of the 17th century. Yet, it wasn't until 1820 that the active ingredients of the cinchona bark were isolated by Pelletier and Caventon[47] and designated as the alkaloids quinine (45) and cinchonine (46).



45

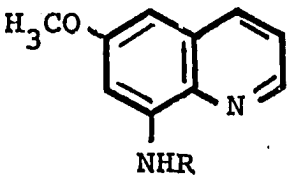


46

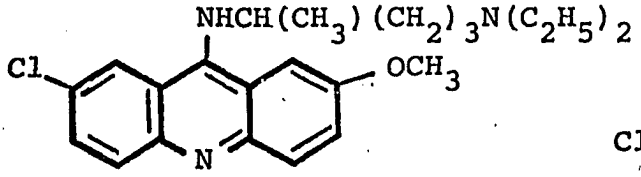
After three centuries of reliance on the cinchona bark as the sole source of antimalarials, a program was initiated for the production of synthetic antimalarials. This program was undertaken by German scientists and was initiated because of the Allied control of the quinine supplies during World War I. This research culminated in the first synthetic antimalarial[48] pamaquine (47) and later in quinacrine (48) which found extensive use prior to World War II.

During World War II a scarcity of quinine and the continued need for effective antimalarial agents led to the development of chloroquine (49). This agent has proven to be one of the most effective antimalarial ever realized. By

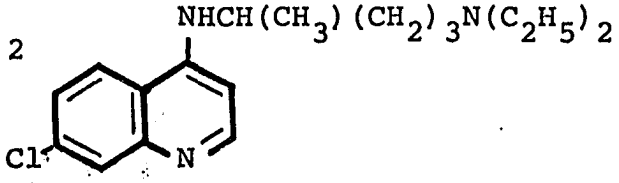
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



	R
47;	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2$
50;	$-(CH_2)_5NHCH(CH_3)_2$
51;	$-CH(CH_3)(CH_2)_3NH_2$



48



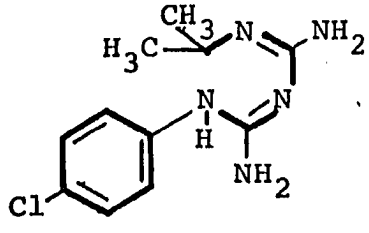
49

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

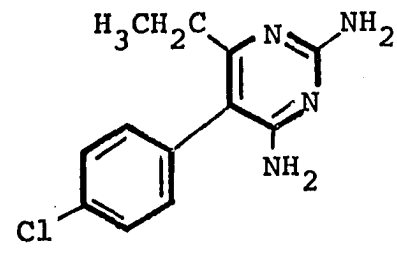
the end of the war, pentaquine (50) and primaquine (51), the least toxic and most effective 8-aminoquinoline antimalarial, had been developed.

Consequently, with the production of so many new and effective synthetic antimalarials, the early 1950's was characterized by a decline of interest in the malaria problem and the premature optimism expressed in the quote cited above. Nevertheless, two factors have initiated renewed interest in the development of new chemical agents efficacious against this disease: (i) the fact that it is necessary in malarial chemotherapy to employ not a single but, in many cases, a variety of drugs to achieve abatement of the disease and (ii) the increasing appearance of various malarial strains resistant to the now currently employed drugs.

This rekindled interest has focused on two major types of antimalarials which are distinguished by their mode of action. The first group, including such compounds as chloroguanide (52) and pyrimethamine (53) are referred to



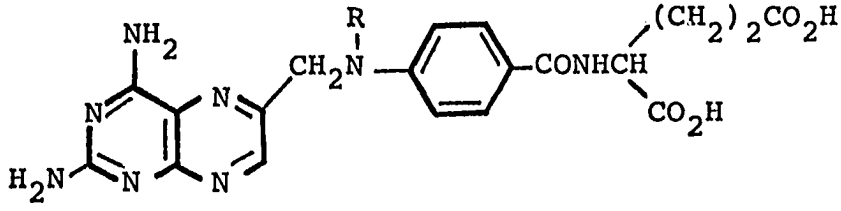
52



53

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

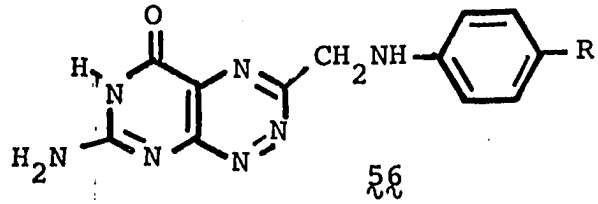
as antifolates because they suppress the p-aminobenzoic acid- folic acid- tetrahydrofolic acid cycle occurring in the malarial parasite. The second group is the quinoline-acridine (47-51) series which inhibit some other, yet unsubstantiated metabolic process. The former approach led Seeger and co-workers[42] to the development of the potent folate antagonists aminopterin (54) and methotrexate (55) an



54; R= H

55; R= CH_3

effective dihydrofolate reductase inhibitor[50]. Furthermore, the research group at the Southern Research Institute[49] has synthesized a number of pyrimido[5,4-e]-*as*-triazines (56) as analogs of pteroic and folic acid. This latter endeavor



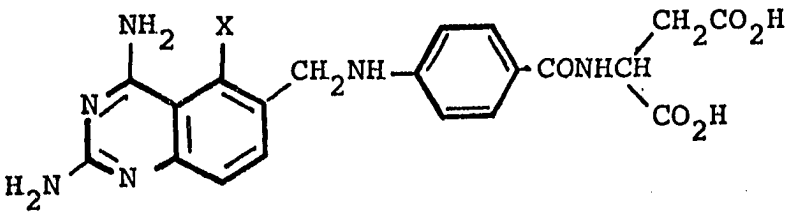
56

R= CO_2H or $CONH(CO_2H)CH_2CH_2CO_2H$

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

represents an example of a bioisosteric substitution in folic acid (-N= for -CH=) in an attempt to more effectively inhibit folic acid reductase.

Elslager and co-workers initially focused their attention on the quinazoline analogs of folic acid (*ie.*, the 5,8-dideazapteridines). It was their assumption that with these analogs the lack of the N-5 in the reduced form would preclude the formation and interconversion of one-carbon units analogous to the processess mediated by the known tetrahydrofolic acid coenzymes 32-35 and 37. The potential of this approach was foreseen by the fact that several classical 2,4-diaminoquinazoline asparaginine analogs[51] (*eg.*, chlorasquin (57a), methasquin (57b) and quinespar (57c))



57a; X= Cl

57b; X= CH_3

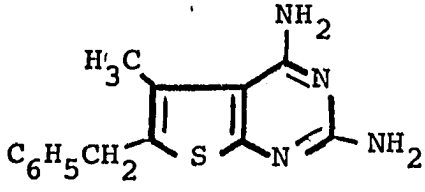
57c; X= H

exhibited potent inhibitory effects[52] against *S. faecalis R*, thymidylate synthetase and dihydrofolate reductase from mammalian and bacterial sources and that 2,4-diamino-6-((3,4-dichlorobenzyl)-amino)quinazoline (58) displayed strong antimalarial activity.[53] The research of the Parke-Davis

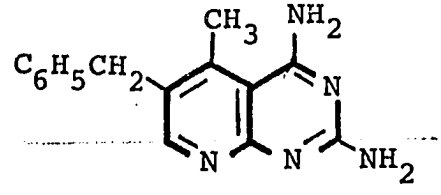
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

team[54] in this area has led to a variety of compounds (59-62) which exhibit potent antimalarial effects against sensitive and drug-resistant strains of malaria. Of particular interest was 2,4-diamino-6-((1,6-dibromo-2-napthyl)-oxy)-quinazoline (63) which proved to possess strong activity at dosage levels of 40 mg/kg and was void of toxicity at the highest levels tested (640 mg/kg).[54]

There has also appeared a considerable amount of interest in the antimalarial potential of 2,4-diaminothieno-[2,3-d]pyrimidines by both Elslager's group[55] and Rosowsky's group.[56] This was based on Roth's report[57] that 2,4-diamino-6-benzyl-5-methylthieno[2,3-d]pyrimidine (64), the thieno-pyrimidine isostere of the potent antibacterial agent 2,4-diamino-6-benzyl-5-methylpyrido[2,3-d]pyrimidine (65), displayed a favorable inhibition ratio against isolated dihydrofolate reductase from bacterial and mammalian sources.



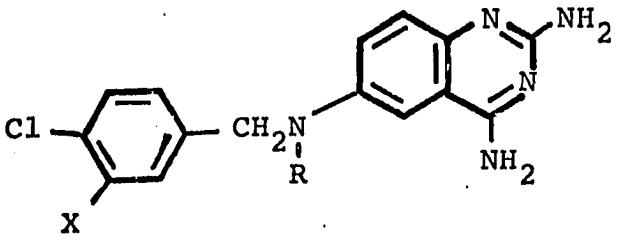
64



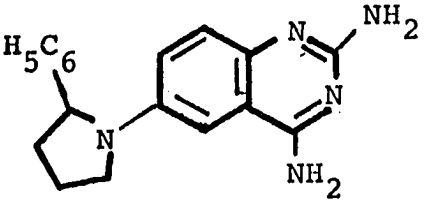
65

However, in their efforts to produce superior antimalarials, Elslager and his co-workers[55] synthesized a number of fused 2,4-diaminothieno[2,3-d]pyrimidines but were able to find

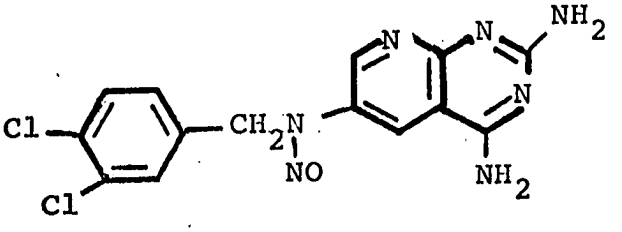
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



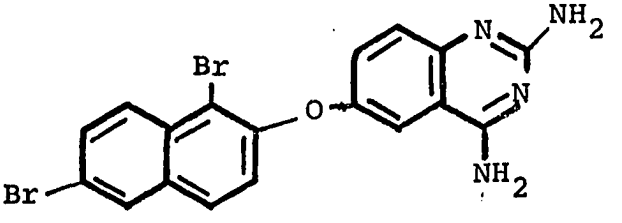
	X	R
58	Cl	H
59	Cl	NO
60	H	$CH(CH_3)_2$



61



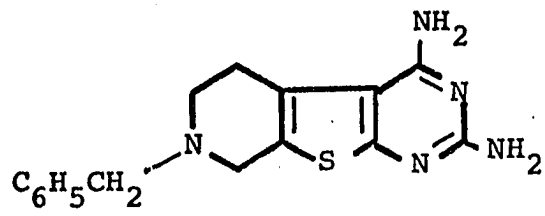
62



63

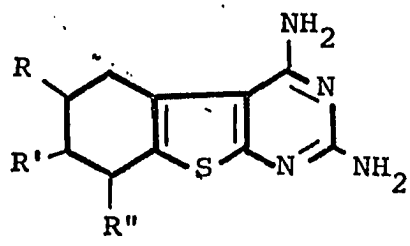
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

significant antimalarial activity in only one of these compounds (*ie.*, 2,4-diamino-7-benzyl-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-d]pyrimidine (66)).



66

On the other hand, the efforts of Rosowsky and his collaborators were more exhaustive and, in some cases, overlapped those of Elslager. In their studies,[56] though, they found a series of fused tricyclic thienopyrimidines and variously substituted 2,4-diamino-5,6,7,8-tetrahydrobenzo [b] thieno[2,3-d]pyrimidines (67) to be inferior to all previously reported antimalarial agents.

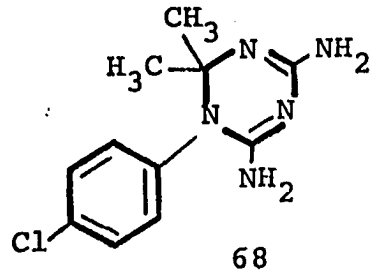


67

A more encouraging development in the area of antifolate antimalarials has arisen from the initial work of Carrington *et al.*,[43] with the chloroguanide (52) metabolite cycloguanil

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(68) which exhibited excellent antimalarial activity. This



68

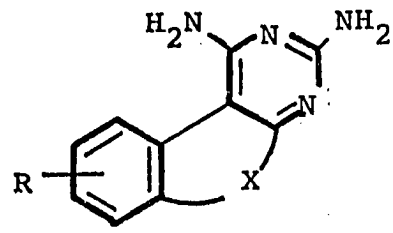
type of small molecule dihydrofolate reductase inhibitor was extended by the work of Hitching and co-workers[41] and culminated with the production of pyrimethamine (53) which possesses extraordinarily remarkable antimalarial effects and has become a widely prescribed clinical antimalarial agent.

Because of its tremendous clinical value and inherent structural simplicity, pyrimethamine (53) has since become the target of extensive structure-activity research. Andre Rosowsky at the Children's Cancer Research Foundation at the Harvard Medical School has been a predominant researcher in this pursuit.[58,59]

Among other factors (*i.e.*, solubility, cell transport and detoxification), the action of pyrimethamine and structurally related antifolates depends, in part, upon their ability to assume a planar or approximately planar configuration at the point of enzyme-inhibitor complex formation.[58] Additional evidence[59] pertaining to this aspect of the structure-activity requirements of pyrimethamine and its

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

analogs has been explored via the tricyclic compounds of general structure 69. In order to more rigorously define



69

the necessary geometrical relationship between the two aromatic rings of pyrimethamine, the *ortho*-position of the phenyl ring is joined to the 6-position of the pyrimidine ring by means of a bridge of varying length and saturation. Systematic modification of the ring bridge in these conformationally rigid analogs generates a family of homologous ring systems suitable for a precise investigation of the effect of molecular geometry on biological activity.

In compound 69 when a -CH=CH- unit comprises the bridge the fully aromatic system results, thus requiring a coplanar structure. On the other hand, with $(CH_2)_n$ units[58] the dihedral angle varies as a function of n, being approximately 0° when n=1, 25° when n=2, and 50° when n=3, with coplanarity becoming increasingly distorted.

The biological testing results indicated that the most effective bridging units were n=2 and -CH=CH-, which were consist nt with the hypothesis that the optimal molecular geometry for inhibition of *S. faecium* is one in which

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the phenyl and pyrimidine rings are coaxial and do not deviate from coplanarity by more than 25°. The coaxial requirement is suggested by the reduced activity of the compound with n=1. In this case, the geometrical requirements of the 5-membered central ring prevent the phenyl and pyrimidine rings from sharing a common axis.

It is apparent from this discussion that the 2,4-diaminopyrimidine ring is a vital structural feature of a number of antimalarial agents. To this end, the research undertaken here involved the synthesis of molecules utilizing the thieno[2,3-b]pyridine moiety as the molecular partner with the bio-significant pyrimidine ring as in 8, which is an unsaturated analog of compound 66 and is related to some of the compounds resulting from Rosowsky's studies[59] with pyrimethamine. The research resulting in such molecular arrays will be presented in the Discussion Section but it should also be noted that this particular research endeavor established new synthetic procedures for the construction of thieno[2,3-b]pyridines which are valuable for future studies concerning the synthesis of thienopyridines isomeric with thieno[2,3-b]pyridine.

B. Antineoplastic Agents

Considerable attention[49] has also been directed toward the use of folic acid based antifolates as antineoplastic agents. Besides aminopterin (54) and methotrexate (55) which were mentioned in the previous discussion, compounds such as

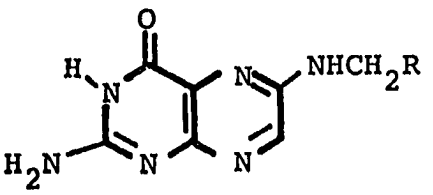
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

isofolic acid (70),[60] 10-deazafolic acid (71),[61] 10-deaza-aminopterin (72),[62] neohomofolic acid (73),[63] and neobis-homofolic acid (74)[63] have all been found to elicit promising biological activity as potential antineoplastic agents. The governing rationale for this research has been the inhibition of the enzymatic action of dihydrofolic acid reductase in rapidly proliferating neoplastic cells.

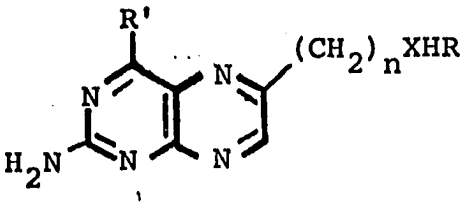
The investigation presented in this thesis focuses on analogs of folic acid (10) and aminopterin (54) with the intention of more rigorously defining the structural relationships which are crucial for biological activity and elucidating a clearer picture of the molecular sites which are responsible for the antifolate activity of these compounds. From this point of view, this study has been directed toward discerning the necessary spatial relationship between the functionalities on the pyrazine and pyrimidine rings of 10 and 54 and the bio-importance associated with fusion of the pyrazine and pyrimidine rings. In pursuit of this objective, the tricyclic analogs of 10 and 54, in which these rings would be separated by atomic bridges which would effectively vary the spatial orientation of the rings and their significant functionalities were envisioned. These planned separated folic acid analogs fall into three classes of compounds which are related by the bioisosteric relationships among the fragments -CH=CH-, -CH=N-, and -S-, as illustrated in Chart V.

The research reported herein is the preliminary work which has been completed toward the tricyc' .c arrays of the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



70



	X	n	R'
71	CH	1	OH
72	CH	1	NH_2
73	N	2	OH
74	N	3	OH

R= p-$C_6H_4CONHCH(CO_2H)CH_2CH_2CO_2H$

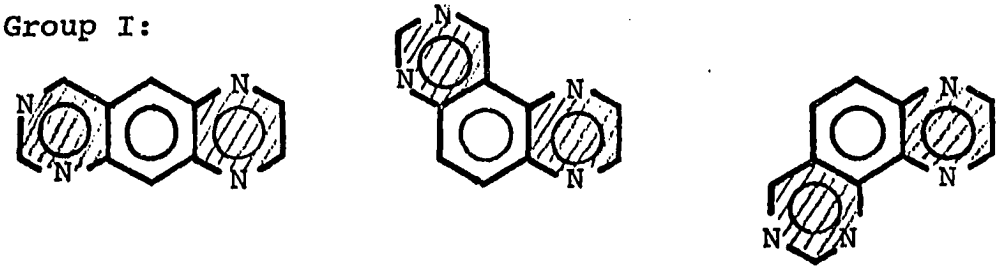
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chart V

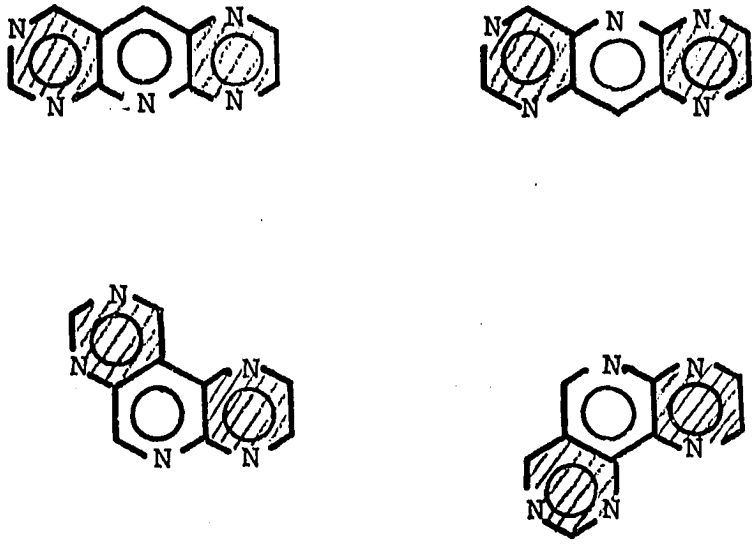
Molecular Framework of Separated

Folic Acid Analogs

Group I:



Group II:



Group III:

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Group III type. Although the goal of this research was simply to develop synthetic pathways to produce such tricyclic arrays, many potentially useful intermediates have been realized toward the actual separated folic acid analogs of this group.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

CHEMICAL BACKGROUND

I. Thienopyridines

Thienopyridines have received considerable attention in the last several years for two very important reasons: (i) their isosteric resemblance to quinoline and isoquinoline renders them important from a pharmacological standpoint, and (ii) from a chemical perspective, the presence of a ring susceptible to electrophilic substitution (thiophene) and a ring vulnerable to nucleophilic substitution (pyridine) which makes selective chemical functionalization on the individual rings a theoretical possibility.

There are six isomeric thienopyridines (75-80), Chart VI) and all of them have been prepared. These six isomers are paired in the presentation below by the similarities based on which face of the pyridine ring the thiophene ring is fused. The syntheses of the members of each of these pairs are usually similar. The discussion presented here will be concerned with only compounds 75-78 since these are the thienopyridines of concern in this thesis. A recent review[64] has appeared and discusses both the synthesis and chemical reactivities of all of the isomeric thienopyridines.

Synthesis of Thieno[2,3-b]- and Thieno[3,2-b]pyridine (75,76)

Both parent systems 75 and 76 have been realized, albeit in low yields, in a high temperature flow system reaction[65] of the appropriate 2- or 3-substituted pyridine in the presence

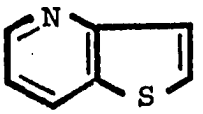
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chart VI

Structures of the Isomeric Thienopyridines

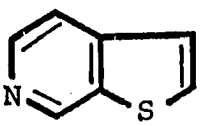
[2,3-b]

75



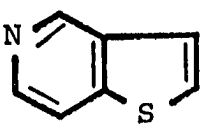
[3,2-b]

76



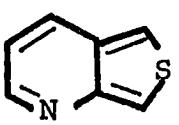
[2,3-c]

77



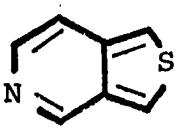
[3,2-c]

78



[3,4-b]

79

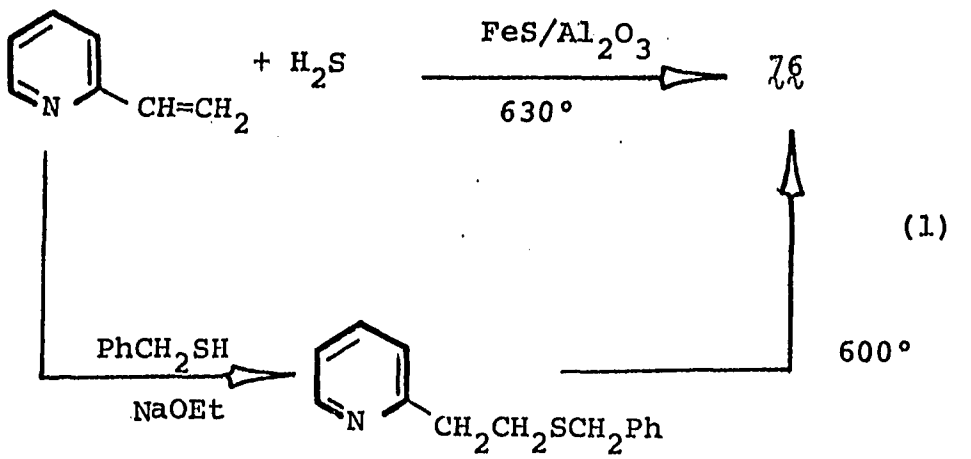


[3,4-c]

80

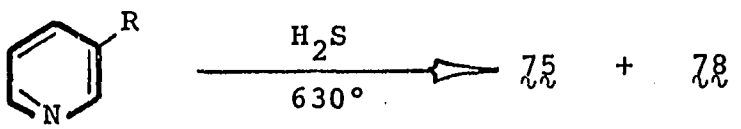
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

of hydrogen sulfide (eq 1). Slightly better yields were



(1)

obtained when the vinylpyridine was first reacted with benzyl mercaptan[66] and the intermediate benzyl-(2-pyridyl)-ethyl sulfide subjected to pyrolysis. It has also been shown that a variety of 3-substituted pyridines react with hydrogen sulfide[67] under these conditions to give a mixture of 75 and 78 (eq 2).



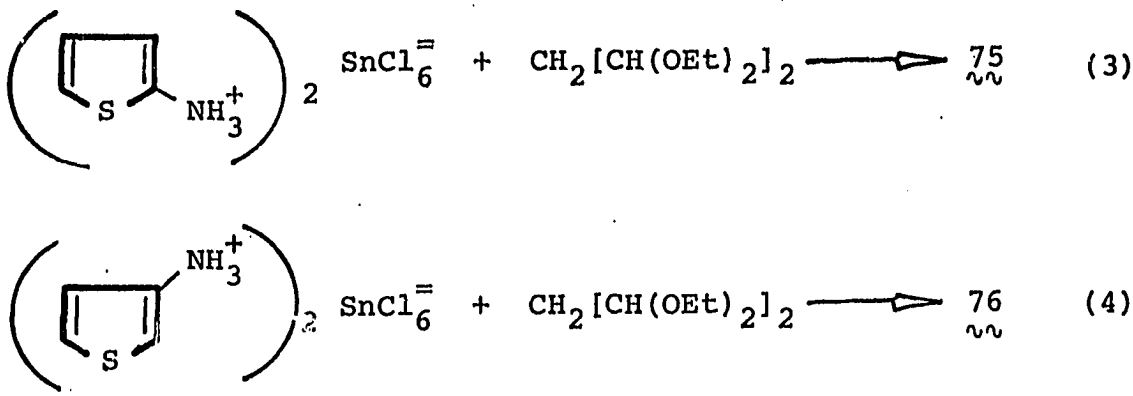
(2)

R= $CH=CH_2$; CH_2CH_3; $CH(OH)CH_3$; $COCH_3$

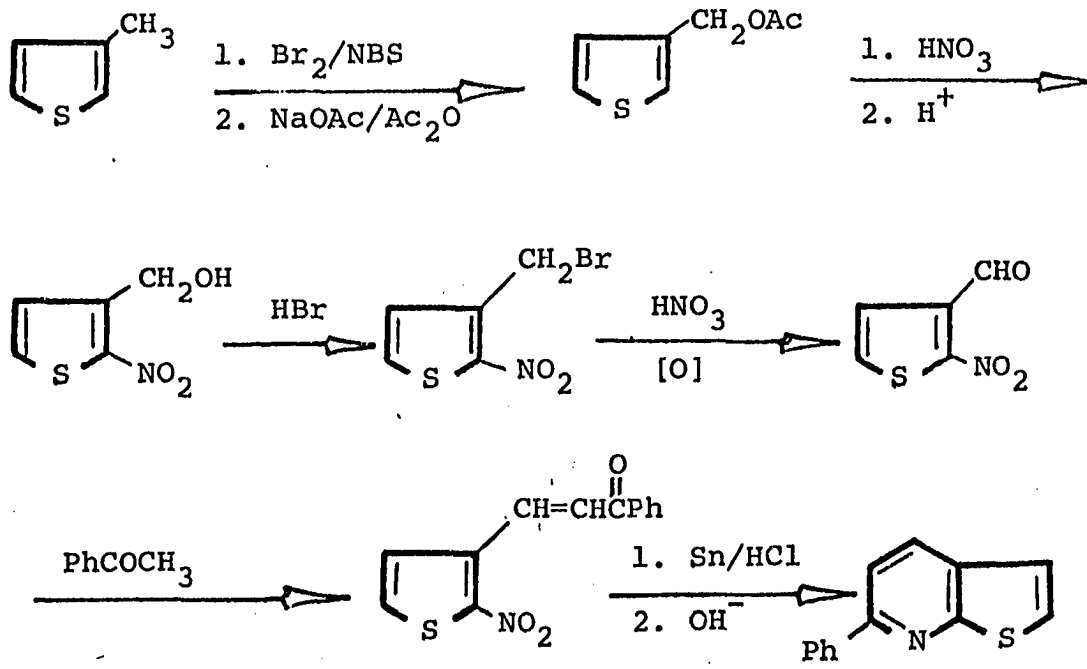
In a higher yielding procedure involving the condensation/cyclization of the requisite 2- or 3-thienylammonium hexachlorostannate with malondialdehyde tetraethyl acetal[68] the parent compounds 75 (eq 3) and 76 (eq 4) were again realized. This reaction has been used successfully with

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

other 1,3-dicarbonyl compounds to produce substituted analogs of 75 and 76.[69-73]

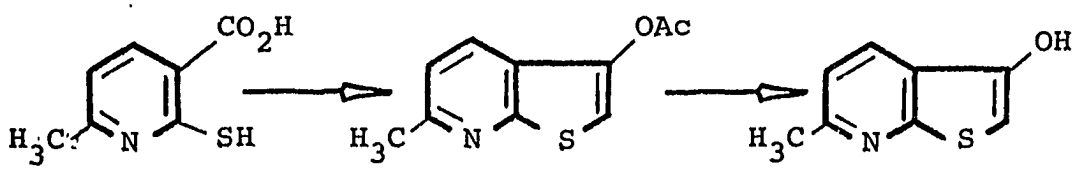


Several somewhat less useful preparations of substituted thieno[2,3-b]pyridines have been reported including the elaborate example shown below[74] commencing with 3-methylthiophene.

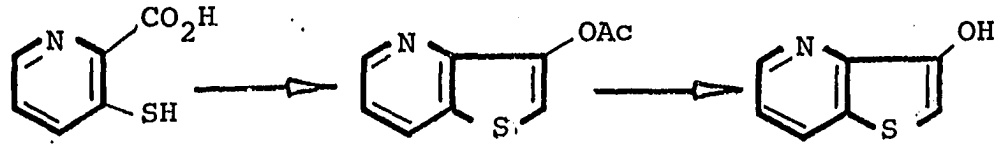


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Finally, it should be noted that various 3-hydroxythienopyridines have been synthesized starting from the appropriate o-mercaptocarboxylic acid derivative of pyridine which is first reacted with chloroacetic acid followed by cyclization with acetic anhydride and finally hydrolyzed. This is illustrated below for the synthesis of 81[71] and 82.[75]



81



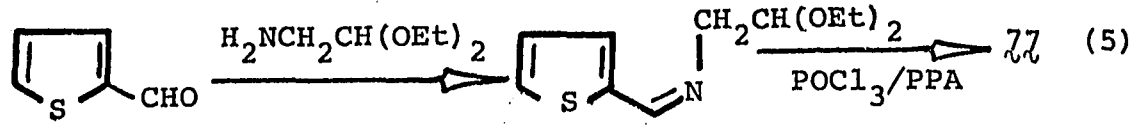
82

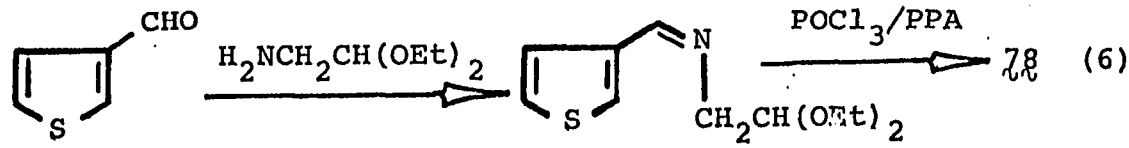
Synthesis of Thieno[2,3-c]- and Thieno[3,2-c]pyridine (77,78)

As mentioned previously,[67] 78 is available from the reaction of 3-vinylpyridine with hydrogen sulfide at elevated temperatures (eq 2). However, a number of more convenient preparations have been reported. By analogy to isoquinoline syntheses, the Pomeranz-Fritz reaction (eq 5 and 6),[76] the Bischler-Napieralski reaction (eq. 7 and 8),[77-80] and the Pictet-Spengler method (eq. 9)[81] have all been employed as routes to 77 and 78.

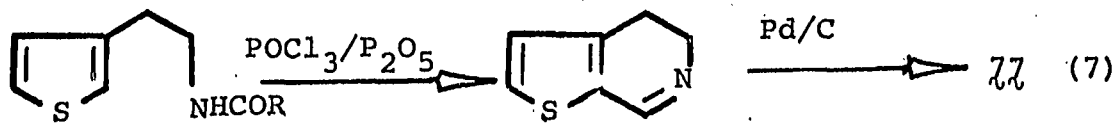
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

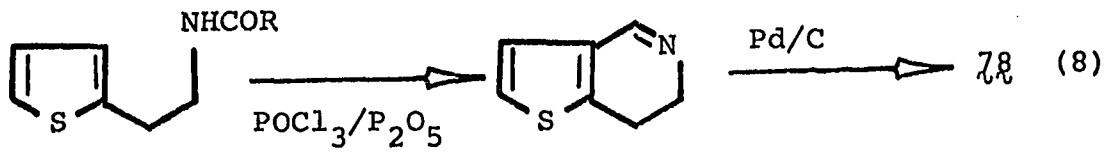
Pomeranz-Fritz Method:



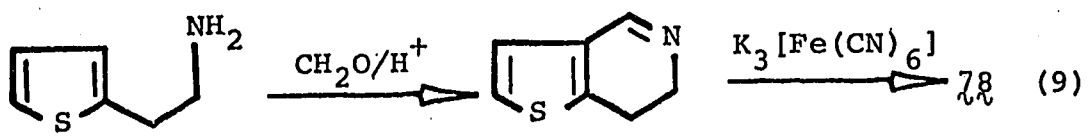


Bischler-Napieralski Method:



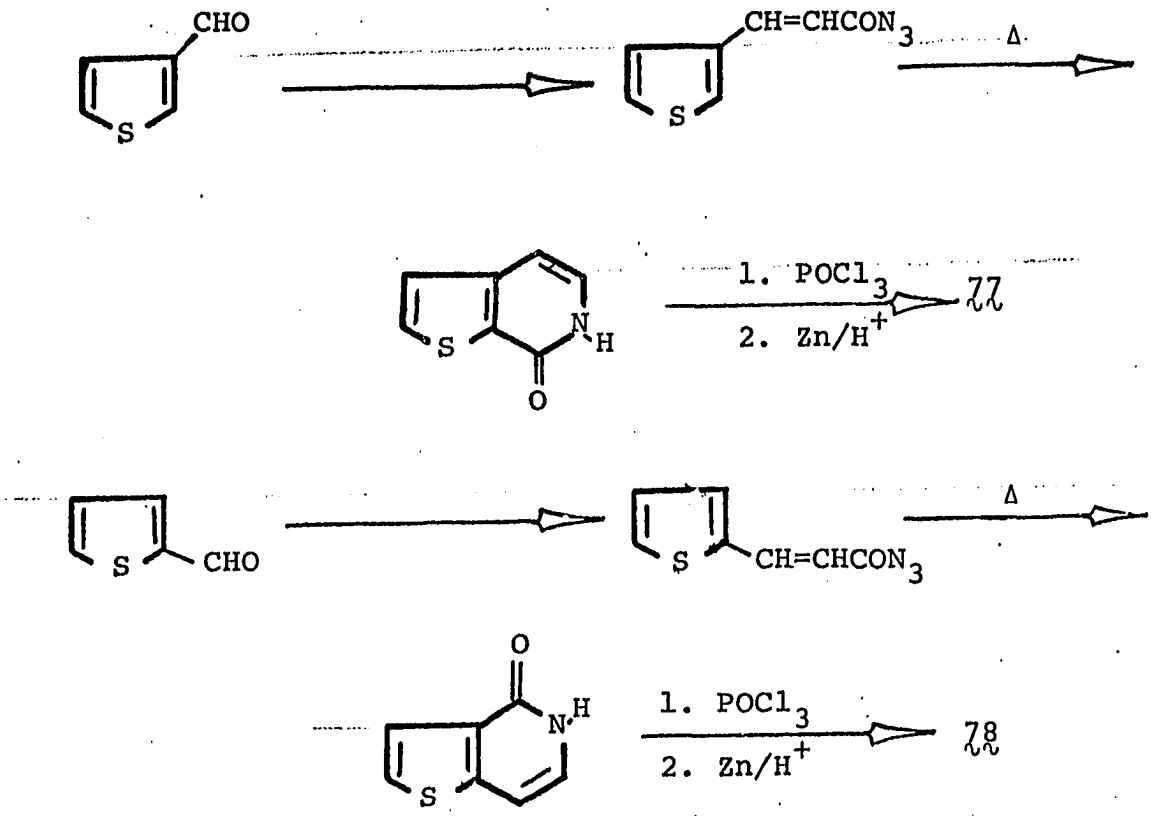


Pictet-Spengler Method:



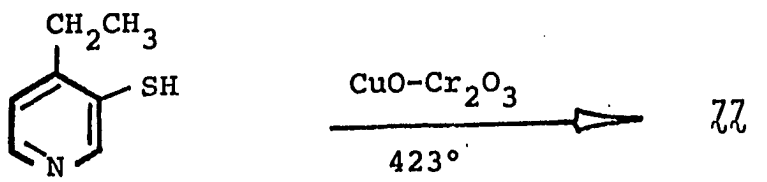
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Compounds 77 and 78 and various substituted derivatives thereof have also been prepared in a process which involves the cyclization of β-thienylvinyl isocyanates to thienopyridones followed by chlorination and reduction[82] as shown below.

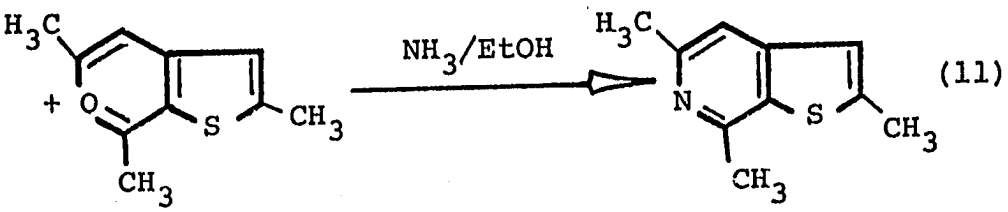


Several less useful preparations have also appeared in the literature. These include the high temperature oxidative cyclization of 4-ethylpyridyl-3-thiol (eq. 10),[83] the treatment of a thienopyrylium salt with ammonia (eq. 11),[84] and the Beckman rearrangement (eq. 12 and 13)[85] to produce precursors to 77 and 78.

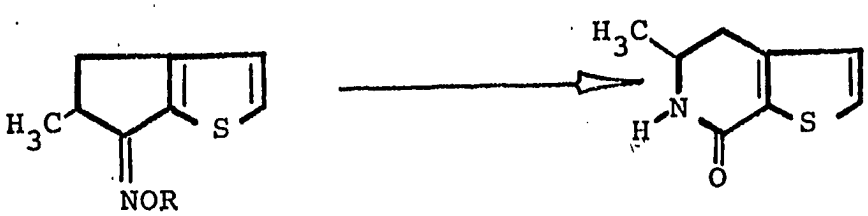
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



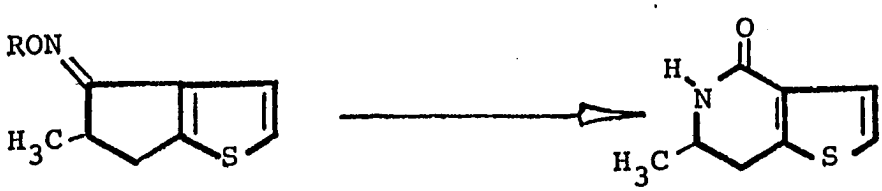
77 (10)



(11)



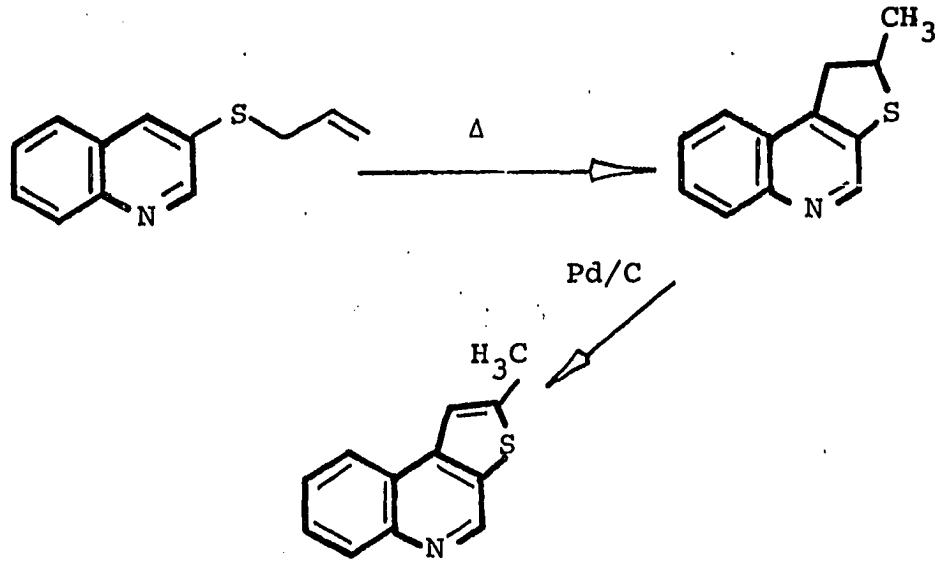
(12)



(13)

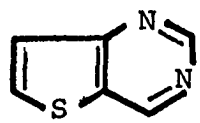
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Finally, in an eloquent approach to a thieno[2,3-c]-quinoline, the [3,3] sigmatropic rearrangement of an aromatic allyl sulfide has been employed[86] as shown below.



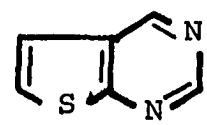
II. Thienopyrimidines

There are three isomeric thienopyrimidines (83-85) and, except for 85, are well documented in the literature. The parent compounds 83 and 84 have been prepared in analogous



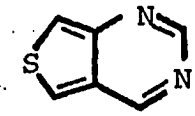
[3,2-d]

83



[2,3-d]

84

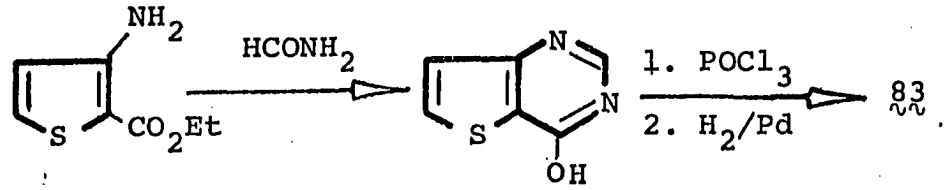


[3,4-d]

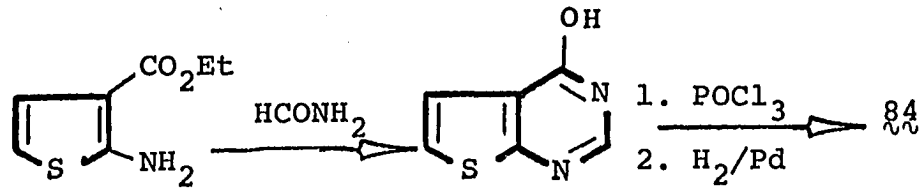
85

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

fashions beginning either with ethyl 3-aminothiophene-2-carboxylate (eq. 14)[87] or ethyl 2-aminothiophene-3-carboxylate (eq. 15).[88]

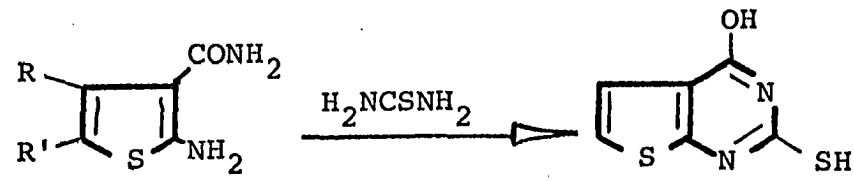


(14)

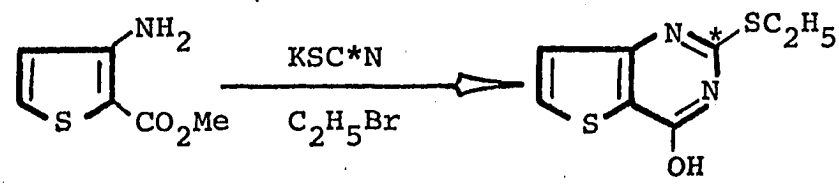


(15)

Various substituted derivatives have been similarly prepared as shown in equation 16[89] and 17.[90] Finally, a



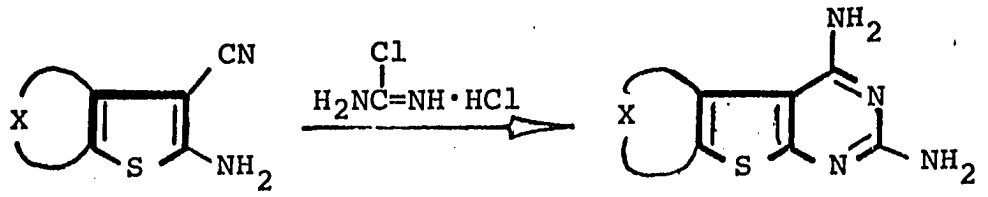
(16)



(17)

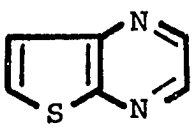
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

variety of 2,4-diaminothieno[2,3-d]pyrimidines[55,56] have been prepared by the reaction of the appropriate 2-amino-3-nitrile thiophene derivative with chloroformamidine.



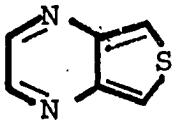
III. Thienopyrazines

There are only two isomeric thienopyrazines (86 and 87), however there are no reports of either of the parent heterocycles in the literature with the exception of the report which resulted from the work presented here.[91] The only



[2,3-b]

86



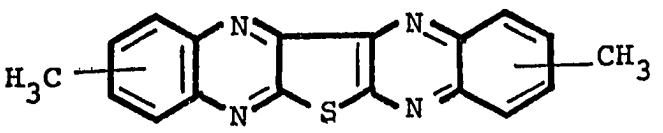
[3,4-b]

87

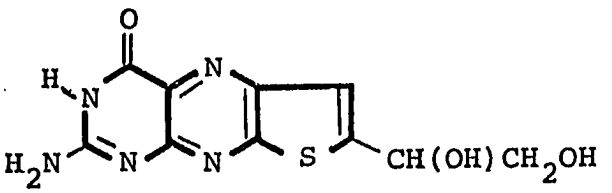
other accounts concerning thieno[2,3-b]pyrazines are those of the photochemical reaction product 88[92] and the urothion derivative 89.[93]

There are a considerably greater number of reports on the synthesis of substituted thieno[3,4-b]pyrazines. Two

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

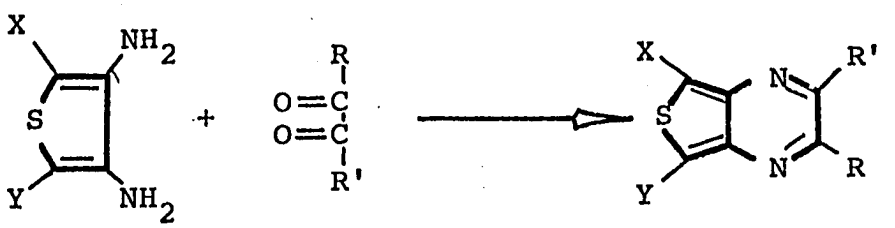


88



89

general procedures are usually employed and involve either the construction of the pyrazine ring via a 3,4-diaminothiophene moiety by reaction of the latter with an α-dicarbonyl compound (eq. 18),[94-96] or the reaction of a 2,3-bis-

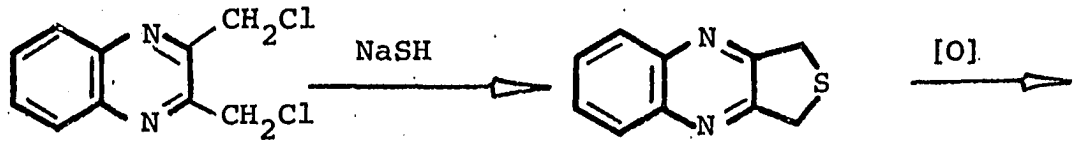


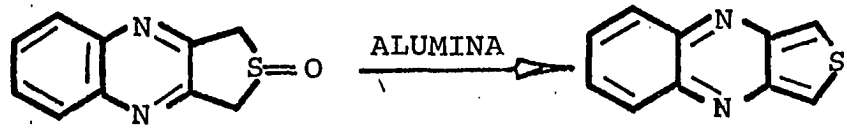
(18)

(chloromethyl)quinoxaline with sodium sulfide followed by oxidation to the sulfoxide and subsequent reductive dehydration by alumina to give 90.[97] The proposed mechanism[97] for

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

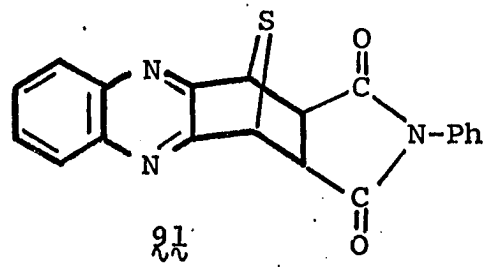
this novel reductive dehydration step involves initial strong adsorption of the sulfoxide on a Lewis acidic site of the alumina surface followed by proton loss from C-1 and C-3 to adjacent basic sites of the catalyst and finally loss of oxide ion to the site of adsorption. Compound 90 was





90

was not isolable but could be trapped with N-phenylmaleimide as the Diels-Alder adduct 91.



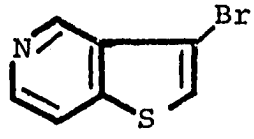
91

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

DISCUSSION OF RESULTS

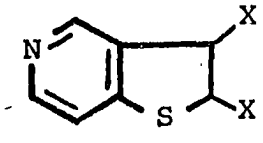
I. Synthetic Approaches to Thieno[3,2-c]pyridines and Analogs of Serotonin

As was stated in the Introduction, the research conducted on the thieno[3,2-c]pyridine isomer was directed specifically toward analogs of serotonin (4) and thus necessitated the synthesis of this ring system with an ethylamine substituent at the 3-position (*i.e.*, 7). Initially it was believed that a halogen at the 3-position as in 92 would serve as the required chemical handle for further elaboration to the desired ethylamine side chain. A survey

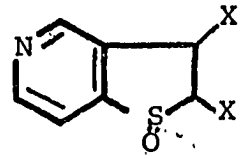


92

of the literature revealed that Gronowitz[81] and Klemm[82] had made in depth studies of the chemistry of the thieno[3,2-c]- and thieno[2,3-b]pyridine ring systems, respectively. Klemm and his co-workers[82] found that halogenation of thieno[2,3-b]-pyridine was more complex than expected due to the formation of a plethora of products including those derived from addition, substitution and oxidation processes (shown below). They were,



addition

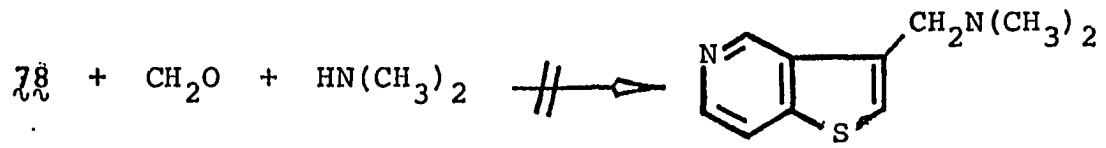


oxidation

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

however, able to effect a respectable yield of 3-bromothieno-[2,3-b]pyridine under explicit and complex conditions. Gronowitz,[81] on the other hand, experienced little difficulty in preparing 3-bromothieno[3,2-c]pyridine under a variety of conditions and in yields of 40-60%. Unfortunately, an attempt to repeat the bromination of 78 was unsuccessful.

An alternative route to an appropriate 3-derivative was thought to be the Mannich reaction on thieno[3,2-c]-pyridine (78), which would produce the Mannich base 93. However, these attempts were similarly fruitless, resulting only in recovered starting material. These latter results

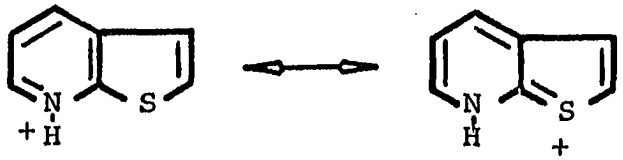


are, however, not surprising in light of the report of Joullié and Dressler[80] on the chemical reactivities of the isomeric thienopyridines. Basing their results on the relative basicities of the isomers, they conclude that the degree of protonation is a function of the basicity of the hetero-aromatic amine[80] and, therefore, dependent on the availability of the lone pair of electrons on the nitrogen atom. Thus, the more basic the system, the more likely it exists in a protonated form. Of the resonance forms available for the protonated isomers (94 and 95), they argue that the *para*-quinoid resonance structure of the thieno[3,2-c]pyridine

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

isomer (94b) would be expected to be more stable than the corresponding orthoquinoid resonance structure available to the thieno[2,3-b]pyridine (95b). The resulting increase

94a 94b



95a 95b

in resonance stability for the thieno[3,2-c]pyridine system allows this isomer to act as a stronger base, resulting in a greater tendency for this system to exist as the protonated species and, hence, lowering its (*i.e.* 78) reactivity toward electrophilic attack at the three position. This argument is congruent with our results but apparently not those of Gronowitz.[81]

In order to circumvent our inability to functionalize the parent heterocycle (78) at C-3, it was deemed necessary to construct an appropriate derivative from simpler molecules. This was pursued by commencing with 3-methylthiophene (96) which was first formylated[98] (Chart VII) by sequential treatment with n-butyl lithium and N,N-dimethylformamide to give 4-methylthiophene-2-carboxaldehyde (97). Knoevenagel reaction[82] of 97 with malonic acid in pyridine/

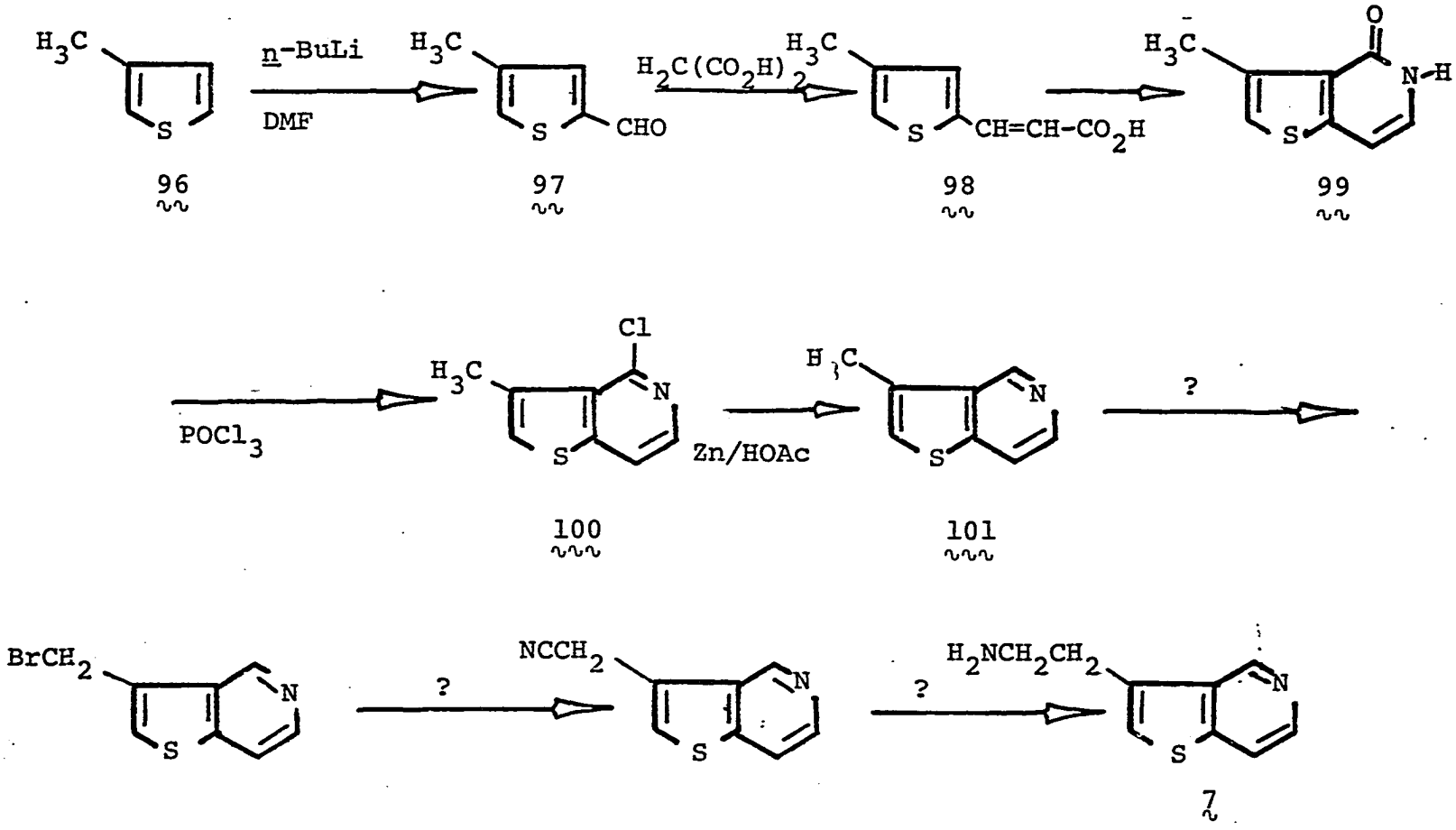
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chart VII

Synthesis of

3-Methylthieno[3,2-c]pyridine

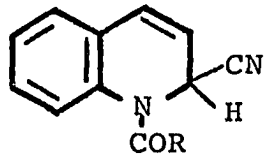


piperidine resulted in 3-[3-(4-methylthienyl)]propenoic acid (98). Reaction of 98 first with ethyl chloroformate followed immediately by sodium azide in acetone at ice-water temperatures resulted in the formation of the acyl azide (IR; 4.68 μ) which, although isolated and found to be stable at room temperature, was not characterized but was subjected to a Curtius rearrangement followed by ring closure to the lactam 99. Chlorination of 99 with phosphorus oxychloride yielded 100 which was converted to 3-methylthieno[3,2-c]-pyridine (101) upon reduction with zinc in glacial acetic acid.

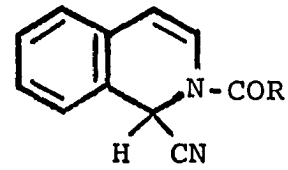
With 101 available, the 3-methyl substituent was anticipated to be an effective chemical handle which could easily be elaborated to the desired 3-ethylamine side chain. Toward this end, allylic bromination of 101 was conducted according to the procedure of Campaigne *et al.*[99] In accordance with this procedure, it was anticipated that the bromomethyl derivative would be unstable and therefore nucleophilic displacement with cyanide ion was carried out on the crude product resulting from the bromination. Unfortunately, even though a couple of displacement procedures were attempted, no product could be isolated which displayed a nitrile absorption in the infrared spectrum. Consequently, further attempts to synthesize 7 were discontinued and it is left to future studies to investigate the synthetic potentials of 101.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Although the parent heterocycle, thieno[3,2-c]-pyridine (78), proved intractable as a synthetic intermediate directed toward serotonin analogs, it was applied in the synthesis of a thieno analog of an isoquinoline alkaloid. The synthesis of quinoline and isoquinoline alkaloids often begins with the corresponding Reissert compounds 102 and 103, respectively.[100-102] Therefore, following the procedure of Popp and Blount[101] for the synthesis of isoquinoline



102



103

Reissert compounds, thieno[3,2-c]pyridine was reacted with benzoyl chloride and potassium cyanide (Chart VIII) to produce 104. The structural assignment for compound 104 was based on the fact that the product contained hydrogen chloride beyond a simple Reissert (*e.g.* 103) structure as was indicated by the elemental analysis and the lack of a nitrile absorption band in the infrared spectrum. Compounds of this type (*i.e.*, 104) have been reported previously[100] although they are not common. The low yields realized for the formation of the Reissert compound 104 can be rationalized by this structure since the work-up of the reaction involves a number of aqueous washings which, if 104 is ionic, would decrease its concentration in the organic solvent and account for its diminished yields.

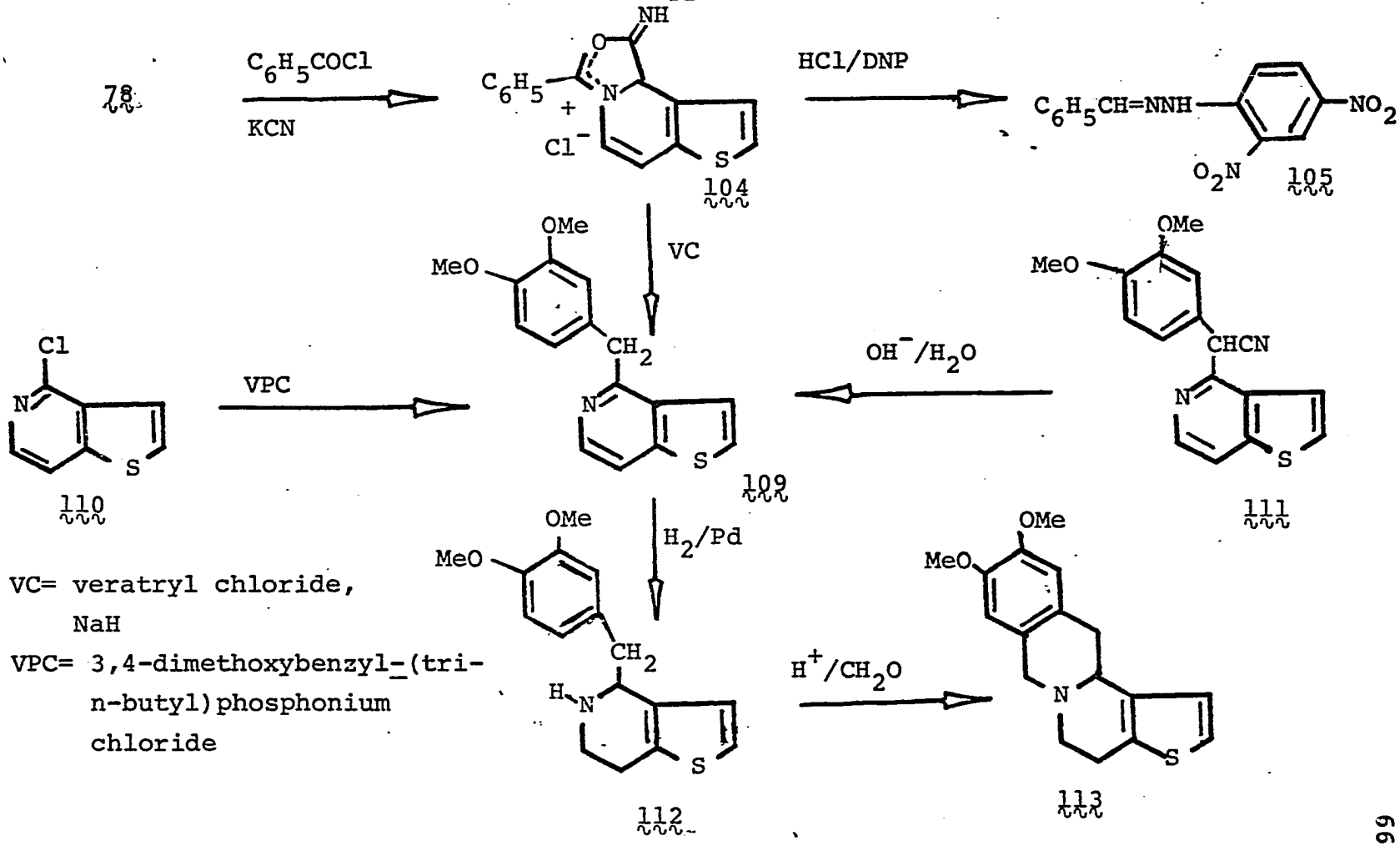
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chart VIII

Reissert Reaction on

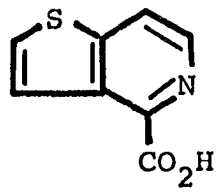
Thieno[3,2-c]pyridine



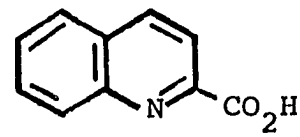
VC= veratryl chloride, NaH

VPC= 3,4-dimethoxybenzyl-(tri-n-butyl)phosphonium chloride

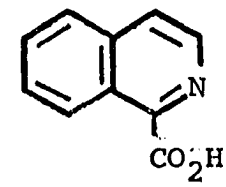
Nevertheless, 104 was shown to undergo a stardard reaction[103] of Reissert compounds. That is, acidic hydrolysis of 104 in the presence of 2,4-dinitrophenylhydrazine resulted in the isolation of the 2,4-dinitrophenylhydrazone of benzaldehyde (105). The other product, although not isolated, was assumed to be thieno[3,2-c]pyridine-4-carboxylic acid (106) by analogy to quinoline-2-carboxylic acid (107) and isoquinoline-1-carboxylic acid (108), the respective products from the acidic hydrolysis of 102 and 103.



106



107

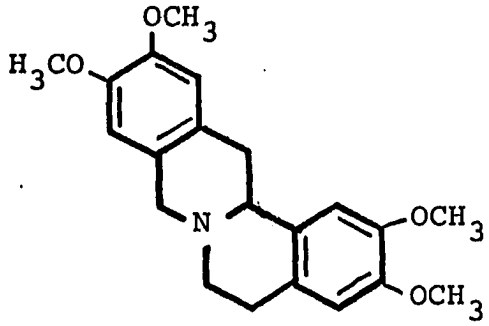


108

Pursuant to the alkaloid objective, 104 was treated first with sodium hydride, then with veratryl chloride and this followed by basic hydrolysis to produce 4-(3',4'-dimethoxybenzyl)thieno[3,2-c]pyridine (109). Compound 109 was also available from an adaptation of Taylor's[104] work with isoquinolines by the reaction of 110 with the ylide derived from the reaction of 3,4-dimethoxybenzylphosphonium chloride with n-butyl lithium. This compound (109) has been synthesized previously via the sodium hydride mediated reaction of 4-chlorothieno[3,2-c]pyridine (110) with 3,4-dimethoxyphenylacetonitrile[105] to give 111 which, after basic hydrolysis, produced 109.

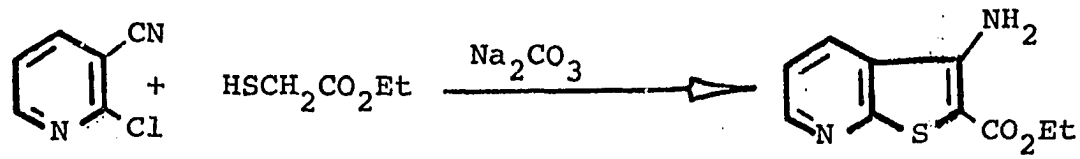
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Catalytic hydrogenation of 109 resulted in the reduction of the pyridine moiety to the tetrahydro derivative 112 which was not characterized but reacted with formaldehyde under acidic conditions[102a] to produce 113 which can be considered to be an analog of the benzylisoquinoline alkaloid[106] papaverine (114), one of the principle alkaloids of opium.



114

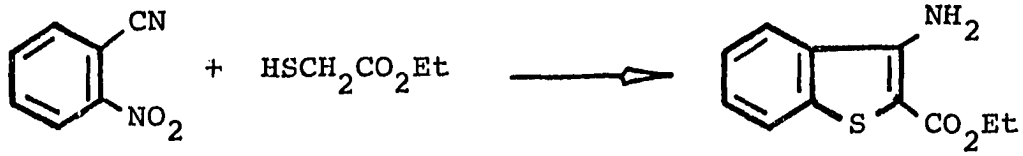
Concurrent with this research, a great deal of experimentation was conducted on the isomeric thieno[2,3-b]-pyridine system in order to develop model procedures which might be adapted toward the studies with the thieno[3,2-c]-pyridine ring system. It has been found[107] that a strategically substituted thieno[2,3-b]pyridine (116) can be efficiently prepared by the sodium carbonate mediated reaction[108-109] of 2-chloro-3-cyanopyridine (115)[110] with ethyl α-mercapto-acetate.



115 116

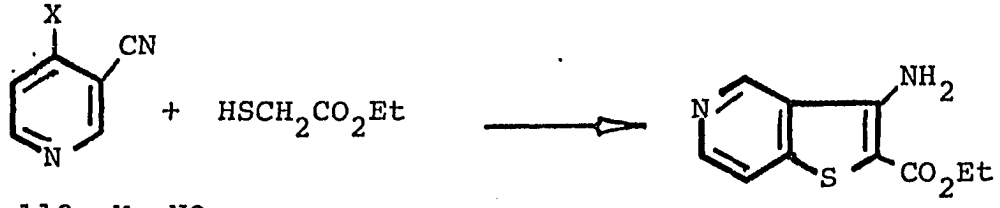
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

This approach to the construction of a thiophene ring has proven to be versatile in this laboratory for the synthesis of thieno[2,3-b]pyrazines (*vide infra*) and benzo[b]thiophenes. In the latter case an acceptable starting material has been shown to be o-nitrobenzonitrile (117).[109]



117

Although not studied here, this approach should be applicable to the synthesis of the isomeric thieno[3,2-c]-pyridine ring system by reacting either 4-nitro-3-cyano-pyridine (118) or 4-chloro-3-cyanopyridine (119) under analogous conditions. This procedure would yield ethyl



118; X= NO_2

119; X= Cl

120

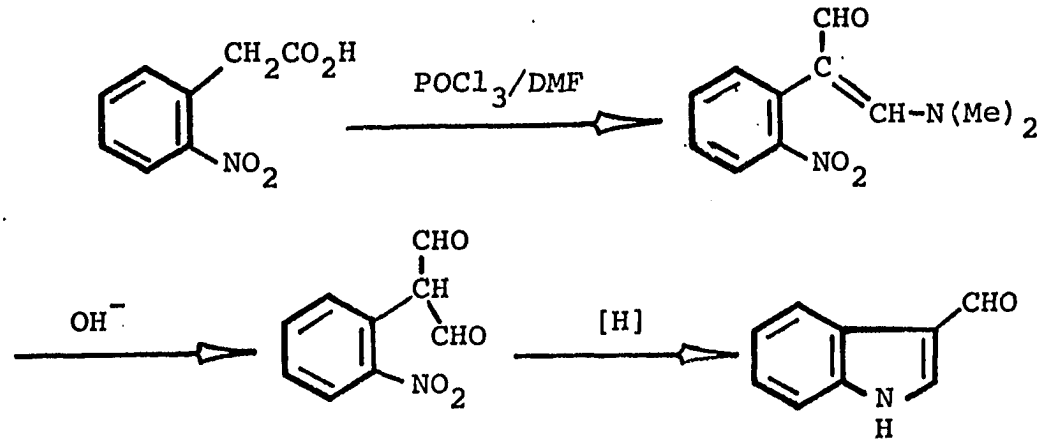
3-aminothieno[3,2-c]pyridine-2-carboxylate (120), a potentially useful intermediate in future studies on the synthesis of 7 or related derivatives.

The search for alternate procedures for the synthesis of 7 resulted in a great deal of frustrating chemistry but the fundamental rationale behind this approach is chemically

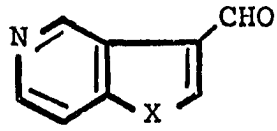
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

sound and worthy of discussion at this time.

An alternate approach originates from the observation that indole-3-carboxaldehyde can be prepared from the corresponding o-nitrophenylacetic acid by the procedure[111] shown below. If the properly substituted 3-pyridylacetic



acids were available, application of this procedure to the synthesis of 121, 122, and 123 could be envisioned.



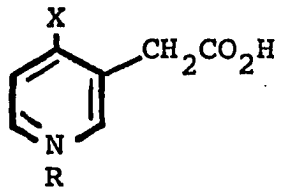
121; X= NH

122; X= S

123; X= O

It was believed that 4-nitro-3-pyridylacetic acid (124) or 4-chloro-3-pyridylacetic acid (125) would represent proper starting materials for such a synthesis. A potential precursor to 124, 4-nitro-3-pyridylacetic acid 1-oxide (126) has been reported,[112] however numerous attempts to reproduce

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



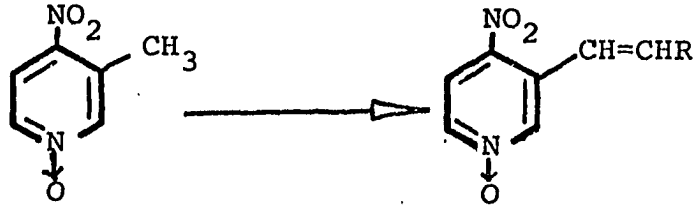
124; X= NO_2, R= no substitutent

125; X= Cl, R= no substituent

126; X= NO_2 R= O

this synthesis failed, and thus considerable effort was directed at alternate routes to 124. For example, it has been shown[113] that 3-picoline condenses with esters to give ketones (eq. 19). Furthermore, Taylor[114] has shown that 4-nitro-3-picoline-1-oxide condenses readily with aldehydes to give a variety of 4-nitro-3-styryl-pyridine-1-oxides (eq. 20). Unfortunately, all of our

(19)

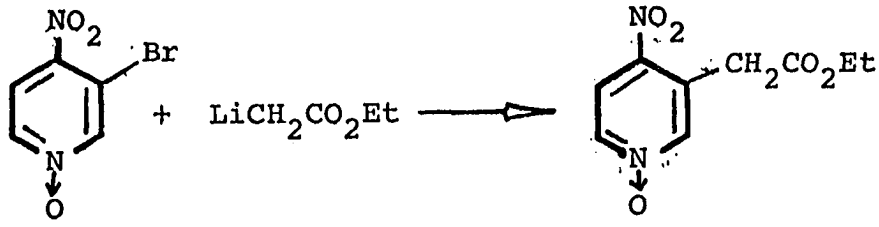


(20)

attempts, based on these results, to convert 4-nitro-3-picoline-1-oxide or 4-nitro-3-picoline to the ethyl ester of either 124 or 126 using a variety of bases (sodium ethoxide, sodium hydride, sodium diisopropylamine or diisopropylethylamine) and either ethyl chloroformate or diethyl carbonate as the condensing agents resulted only in recovery of starting materials. Additionally, nitration of 3-pyridylacetonitrile 1-oxide was unsuccessful as was

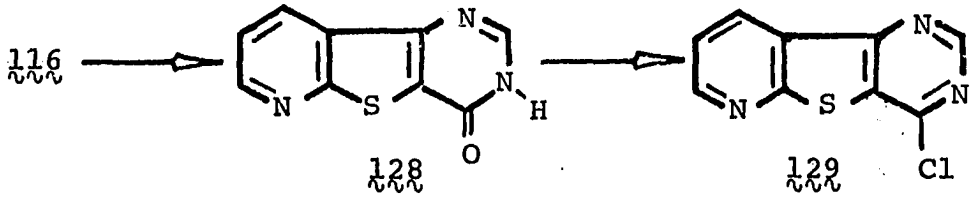
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

nitration of ethyl 3-pyridylacetic acid 1-oxide. This approach was, consequently, abandoned, but in view of its synthetic potential it should be reinvestigated at a future date. One possible modification to 124 which might prove beneficial would be to react 4-nitro-3-bromopyridine-1-oxide with the lithium salt of ethyl acetate[115] to give the ethyl ester of 126.



II. Synthetic Approaches to Thienopyridine Analogs of Antifolate Antimalarials

Pursuant to the synthesis of tricyclic arrays incorporating a thienopyridine as the molecular partner for the bio-significant pyrimidine ring, ethyl 3-aminothieno[2,3-b]-pyridine-2-carboxylate (116) proved to be a versatile starting material. Reaction of 116 with formamide resulted in the pyrimidin-4-one (128) which was readily chlorinated by the action of phosphorus oxychloride to give 129. This



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

4-chloro derivative proved to be very useful for many synthetic conversions as summarized in Chart IX. The chloro-substituent was extremely labile, being readily replaced by a variety of nucleophiles. For example, reaction of 129 with (a) sodium methoxide resulted in the 4-methoxy derivative 130, (b) with thiourea the 4-mercapto derivative 131 formed, and (c) with hydrazine or N-methylhydrazine gave 132 and 133, respectively. Compound 132 was readily converted to the fused *s*-triazolo derivative 134 by treatment with formic acid, or to the fused tetrazolo derivative 135 by diazotization in acetic acid. Compound 135 was also available directly from 129 by treatment with sodium azide.

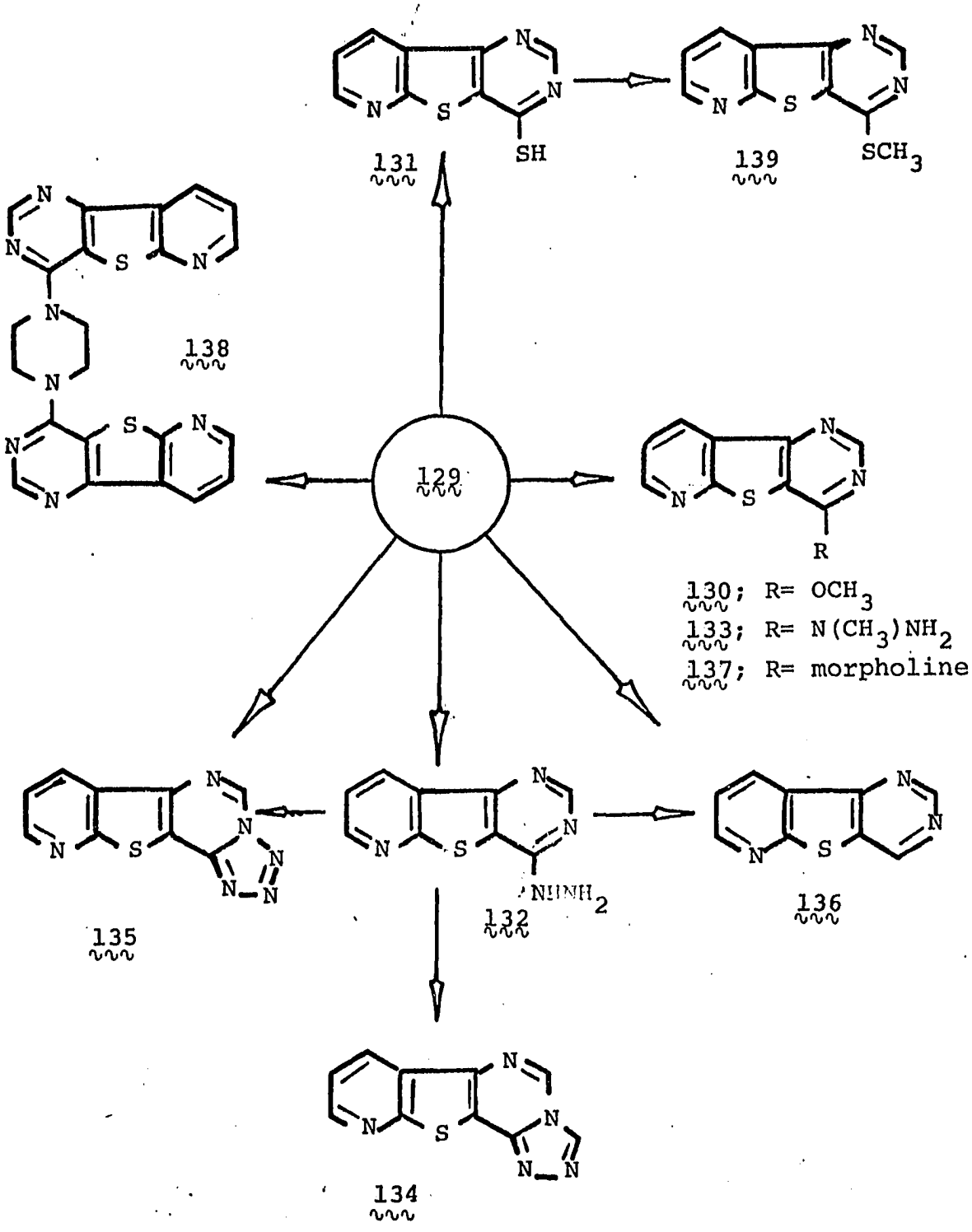
Both 129 and 132 were shown to be precursors to the parent unsubstituted tricyclic heterocycle, pyrido[3',2':4,5]-thieno[3,2-d]pyrimidine (136). The route from 129 to 136 involved the atmospheric hydrogenation of 129 using palladium on charcoal as the catalyst, whereas 136 was produced from 132 by treating a sodium ethoxide/ethanol solution of the latter with oxygen.

Two other nitrogen nucleophiles were reacted with 129: morpholine yielded coupound 137 and piperazine produced compound 138. The versatility of replacing this substituent with nucleophiles indicates that it might be possible to replace the chlorine atom with an aminoalkylamine to give compounds analogous to some of the antimalarial agents mentioned earlier (*e.g.*, 48-51).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

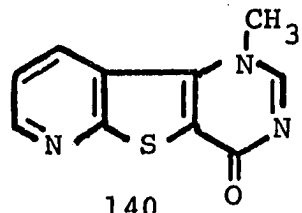
Chart IX

Reactions of Compound 129

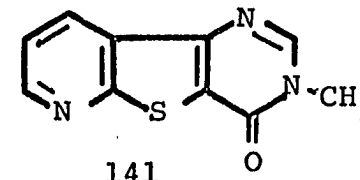


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The alkylation of the 4-mercapto derivative 131 with dimethylsulfate and employing sodium ethoxide as the base resulted in S-alkylation (*i.e.*, 139) as the sole product. In contrast, similar alkylation conditions failed to produce 130 from 128, but instead yielded a product which retained the characteristic infrared carbonyl absorption for the lactam. The alkylated product could therefore have been either 140 or 141, and, in fact, was proved to



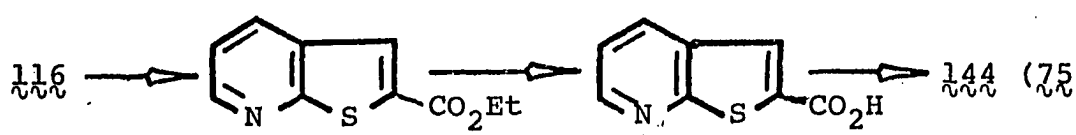
140



141

be 141 unequivocally by the independent synthesis of 141 by reacting 116 with N-methylformamide. This product was shown to be identical to that which was isolated from the alkylation of 128.

With compound 116 available, the synthesis of the unsubstituted thieno[2,3-b]pyridine (144, *i.e.*, 75) was undertaken to improve on the overall yields to 144 which have been reported in the literature.[65-68] Diazotization of 116 with sodium nitrite in sulfuric acid, followed by reduction with hypophosphorus acid gave 142, which was readily saponified by the action of alcoholic potassium

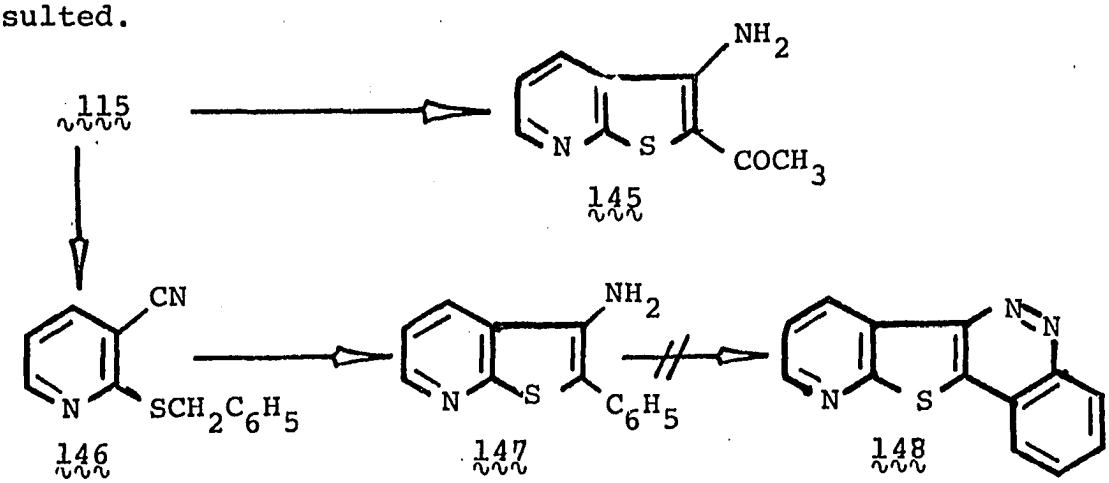


142 143

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

hydroxide to give 143. Compound 143 was effectively decarboxylated to give 144 in an overall yield of 26% starting from 116.

Other compounds realized in the thieno[2,3-b]pyridine series included 145 and 147. When 115 was reacted with mercaptoacetone, 3-amino-2-acetylthieno[2,3-b]pyridine (145) resulted. The similar reaction of 115 with benzyl mercaptan did not proceed to the ring closed product 147, but instead formed 2-benzylmercapto-3-cyanopyridine 146. However, ring closure to 147 was effected by subsequent treatment of 146 with sodium ethoxide in ethanol. Attempts to diazotize 147 in the hopes of forming 148 failed and only intractable tars resulted.



During the course of this investigation, but for other purposes, it was desired to have available 3-hydroxythieno-[2,3-b]pyridine (154; Chart X), furthermore there exists an apparent controversy in the literature concerning this compound.[116] Consequently, the synthesis of 154 was pursued starting from nicotinic acid (149). Peracetic acid oxidation of 149[117] readily produced nicotinic acid 1-oxide (150)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chart X

Synthesis of 3-Hydroxythieno[2,3-b]pyridine

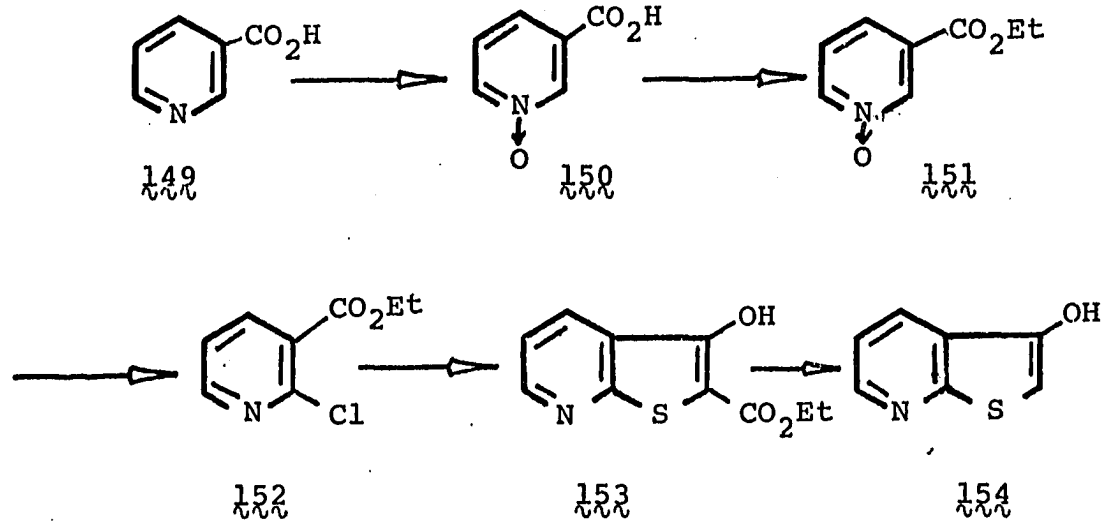
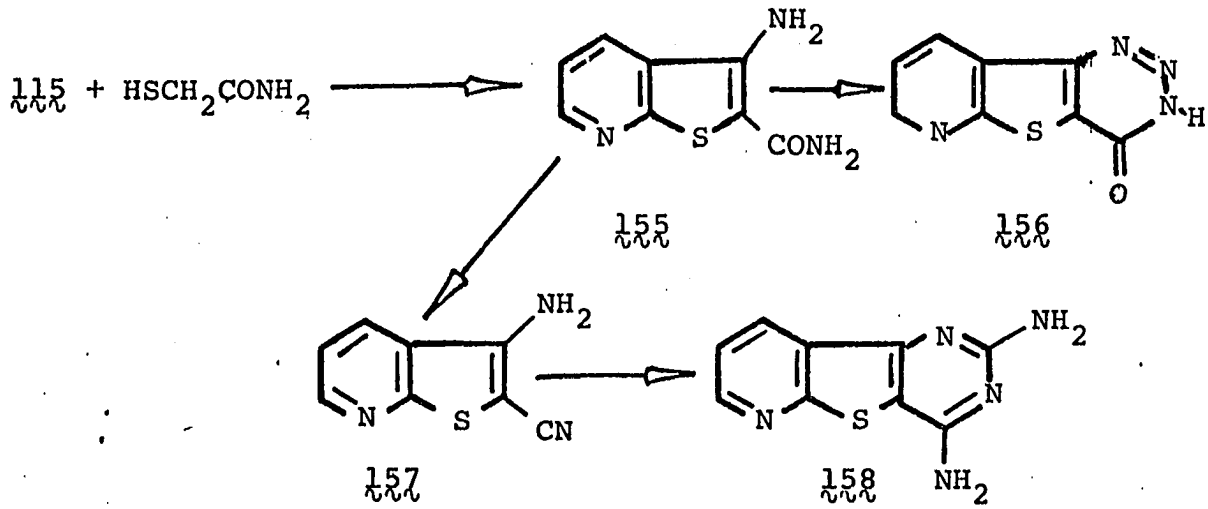


Chart XI

Synthesis of

2,4-Diaminopyrido[3',2':4,5]thieno[3,2-d]pyrimidine



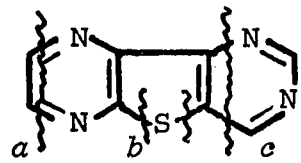
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

which was subsequently esterified[117] with ethanol saturated with gaseous hydrochloric acid to yield 151. Chlorination of 151 with phosphorus oxychloride and phosphorus pentachloride produced 152 which, when reacted with ethyl α-mercaptoacetate in the usual manner, resulted in 153. Treatment of 153 with refluxing dilute sulfuric acid produced the desired compound 154.

Finally, all attempts to convert the ester function of 116 to the amide 155 (Chart XI) were unsuccessful. Nevertheless, 155 was readily available from the reaction of 115 with α-mercaptoacetamide. Diazotization of 155 resulted in the *v*-triazinone 156, whereas phosphorus oxychloride dehydration gave 3-amino-2-cyanothieno[2,3-b]pyridine (157). *Ortho*-amino-nitriles are often used for the formation of 2,4-diamino-pyrimidine rings (*vide supra*), and in fact the reaction of 157 with chloroformamidine in diglyme resulted in the formation of 2,4-diaminopyrido[3',2':4,5]thieno[2,3-d]pyrimidine (158), the desired antimalarial derivative.

III. Synthetic Approaches to Thieno-Separated Analogs of Folic Acid.

Pursuant to the synthesis of the parent tricyclic heterocycle 159 as a separated folic acid ring system, there are at least three dislocation points[118] (marked a, b, and c)



159

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

which are available for ring construction. Examination of the requisite precursors which would be necessary for each possible mode of ring formation permits a reasonable decision to be made as to the potential of each as a possible synthetic scheme for the desired compound. For instance, dislocation at 'a' would require a 2,3-diaminothieno[3,2-d]-pyrimidine fragment to be condensed with some α-dicarbonyl compound (*e.g.*, glyoxal). Unfortunately, 2,3-diaminothiophenes are not documented in the literature and there is indication that they would be unstable;[119] therefore this potential route was not pursued. Dislocation at 'b' is likewise unattractive because nucleophilic attack at the C-5 position of a pyrimidine ring is unlikely and/or 5-mercaptopyrimidines are difficult to prepare. Finally, dislocation at 'c' is similarly unattractive since the literature contains no report of the thieno[2,3-b]pyrazine ring 86 (see page 57).

This problem was however circumvented by the stepwise formation of a thiophene ring on a properly substituted pyrazine ring with subsequent construction of the pyrimidine ring. This resulted not only in the desired tricyclic system, but also the first report[91] of compound 86. Thus, condensation of glyoxal with 2-aminomalonamide[120] gave 2-hydroxy-3-carboxamidopyrazine (160). This particular reaction continuously gave unreproducible results until the following observations were made. The stochiometry of the reaction is extremely important requiring the use of glyoxal, sodium bisulfite and 2-aminomalonamide in the explicit ratio of

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1:2:1. Secondly, during the oxidative work-up with aqueous hydrogen peroxide, it is essential that the reaction temperature be maintained at 50° ± 5°. If the exothermic reaction which commences after a slight induction period during the addition of the hydrogen peroxide is quenched completely or if the temperature is allowed to rise unabated beyond the temperature ranges noted above, the yield of the reaction is drastically reduced. If these precautions are adhered to, yields on the order of 60-70% are attainable.

The dehydration/chlorination of 160 with phosphorus oxychloride to give 2-chloro-3-cyanopyrazine (161)[121] is a similarly unattractive reaction. The problem here is the extensive extraction necessary to obtain only moderate yields of the desired product (continuous extraction with diethyl ether gave a higher yield of the crude product but after purification by either distillation or sublimation, only moderate yields were again realized, leaving large amounts of undesired residues).

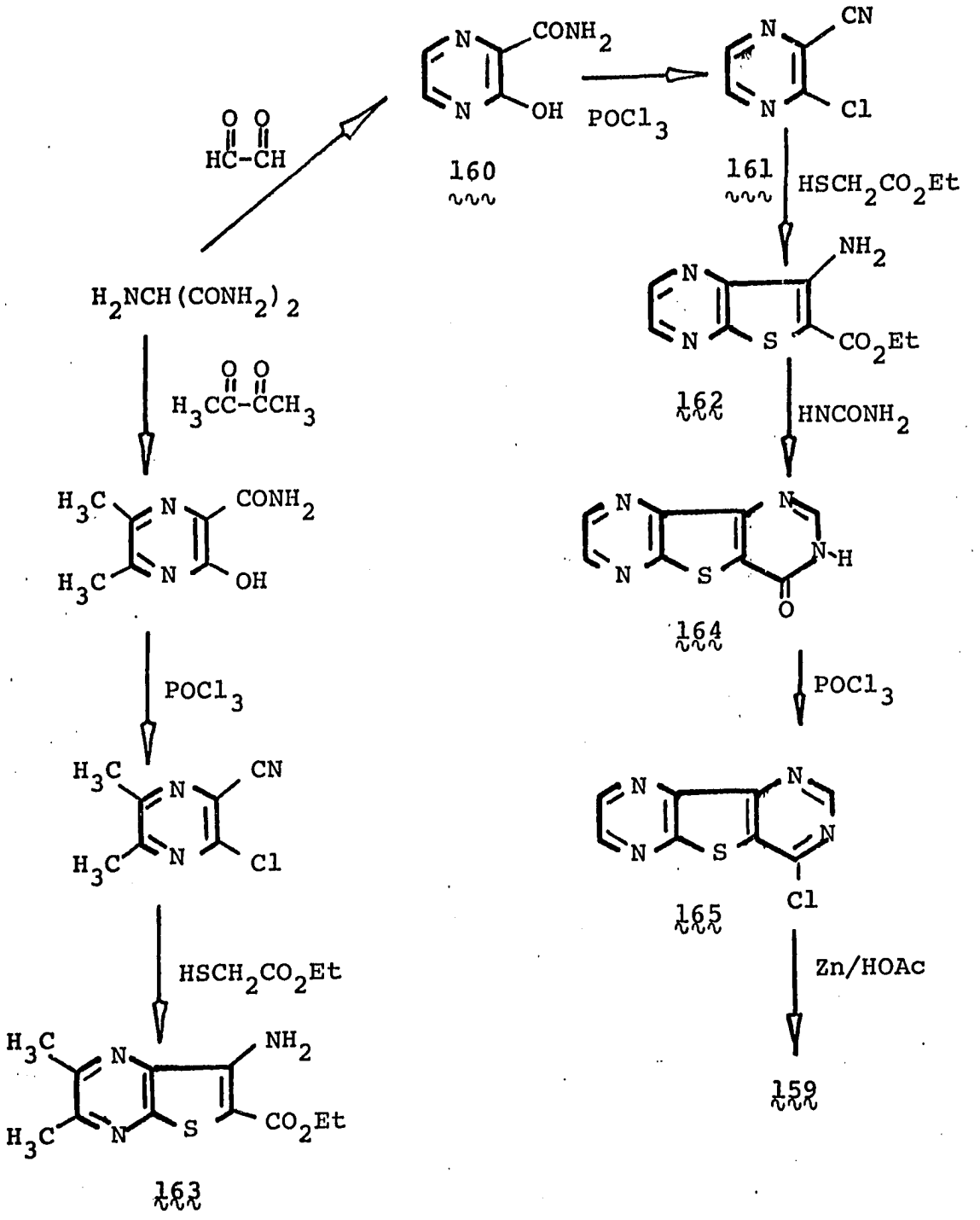
Once 161 was in hand, its sodium or potassium carbonate mediated reaction[108] with ethyl α-mercapto-acetate resulted in ethyl 7-aminothieno[2,3-b]pyrazine-6-carboxylate (162, Chart XII). This sequence could be generalized to allow the incorporation of various functionalities at the C-2 and C-3 positions of the pyrazine ring by the judicious choice of α-dicarbonyl starting material. For example, ethyl 2,3-dimethyl-7-aminothieno[2,3-b]-pyrazine-6-carboxylate (163) is readily realized when

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chart XII

Synthesis of

Pyrazino[2',3':4,5]thieno[3,2-d]pyrimidine



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

biacetyl is substituted for gyloxal in this reaction sequence (Chart XII).

With compound 162 in hand, the completion of the target tricyclic heterocycle 159 was pursued in a straightforward manner (Chart XII). Treatment of 162 with refluxing formamide afforded the pyrimidin-4-one 164. Chlorination of 164 with phosphorus oxychloride yielded the 4-chloro derivative 165, and compound 165 proved to be a fruitful precursor to a variety of molecules including the unsubstituted parent heterocycle 159 by reduction with zinc dust in refluxing glacial acetic acid.

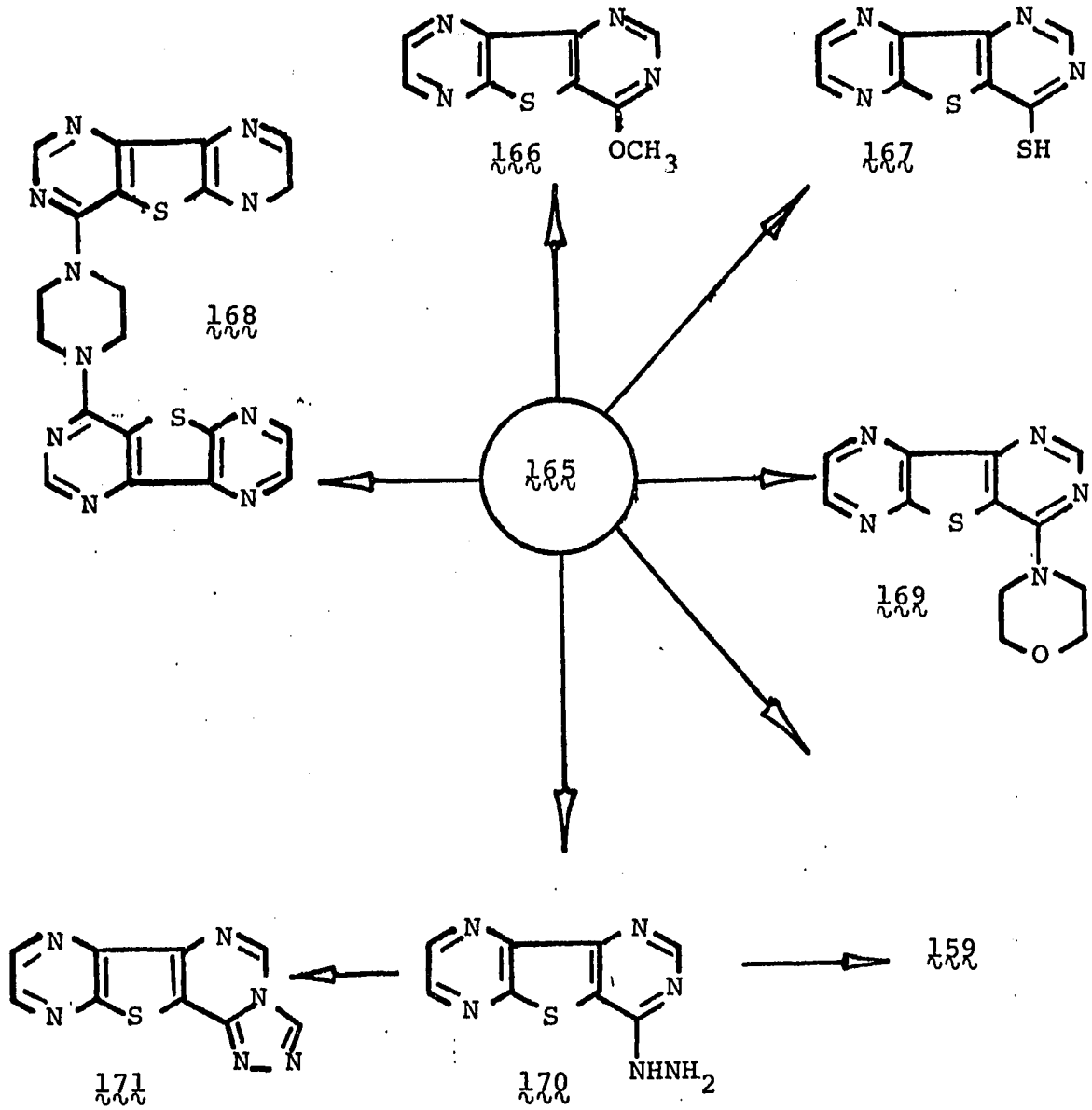
The versatility of compound 165 is shown in Chart XIII. Reaction of 165 with sodium methoxide afforded the 4-methoxy derivative 166 while treatment with thiourea resulted in the 4-mercapto derivative 167. Both compounds 164 and 167 were, however, found to be intractable to simple base promoted methylations. The 4-chloro substituent of 165 was also readily replaced with piperazine, morpholine, and hydrazine to give 168, 169, and 170 respectively. The latter product (*i.e.*, 170) was converted to (i) the fused *s*-triazole 171 on treatment with formic acid and (ii) the parent heterocycle 159 by bubbling oxygen through a sodium ethoxide/ethanolic solution of 170.

A further interesting reaction was that which occurred when the amino acid 172 (available from 162) was refluxed with acetic anhydride (Chart XIV) to give the 2-methyl-oxazinone 173. Reaction of 173 with ammonium hydroxide

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chart XIII

Reactions of Compound 165



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

converted the oxazine ring to the pyrimidin-4-one 174, which is a 2-substituted derivative of 159.

Preparation of the fused 2,4-dioxo-pyrimidine ring system was next pursued. To this end it was necessary to prepare 7-aminothieno[2,3-b]pyrazine-6-carboxamide (175, Chart XIV). This was accomplished by the reaction of 161 with α-mercaptoacetamide to give 175. Dehydration of 175 with either phosphorus oxychloride or phosphorus pentoxide gave 7-amino-6-cyanothieno[2,3-b]pyrazine (176), a compound of significant potential (*vide supra*). The desired ring system (*i.e.*, a fused 2,4-dioxopyrimidine ring) was successfully constructed either (i) by fusion of 172 with urea or (ii) the reaction of 175 with ethyl chloroformate in pyridine. This is a potentially valuable intermediate with regard to the separated folic acid derivatives of the Group III type (see page 45).

In an attempt to prepare the heretofore unknown 86, compound 162 was saponified with alcoholic potassium hydroxide to give 7-aminothieno[2,3-b]pyrazine-6-carboxylic acid (172). Decarboxylation of this compound in the usual manner with copper in quinoline was deemed unattractive because of the difficulties that were foreseen in the isolation of a nitrogenous product. Usually the remnants of quinoline are removed by extraction with dilute mineral acid and in this case there would be no selectivity in removing quinoline in preference to the desired product. Since effervescence was noted when the melting point of 172 was taken, a simple thermal

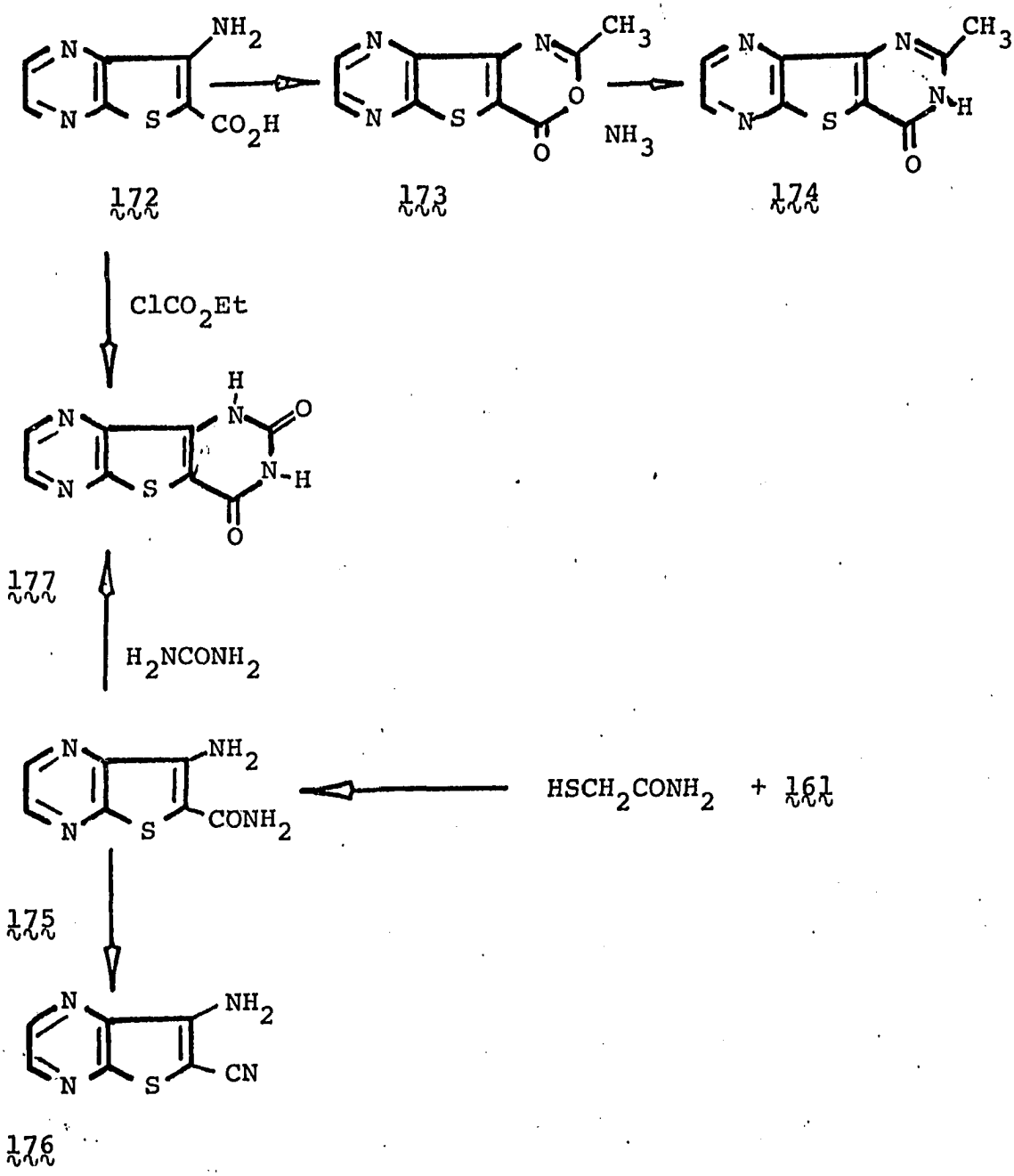
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chart XIV

Synthesis of

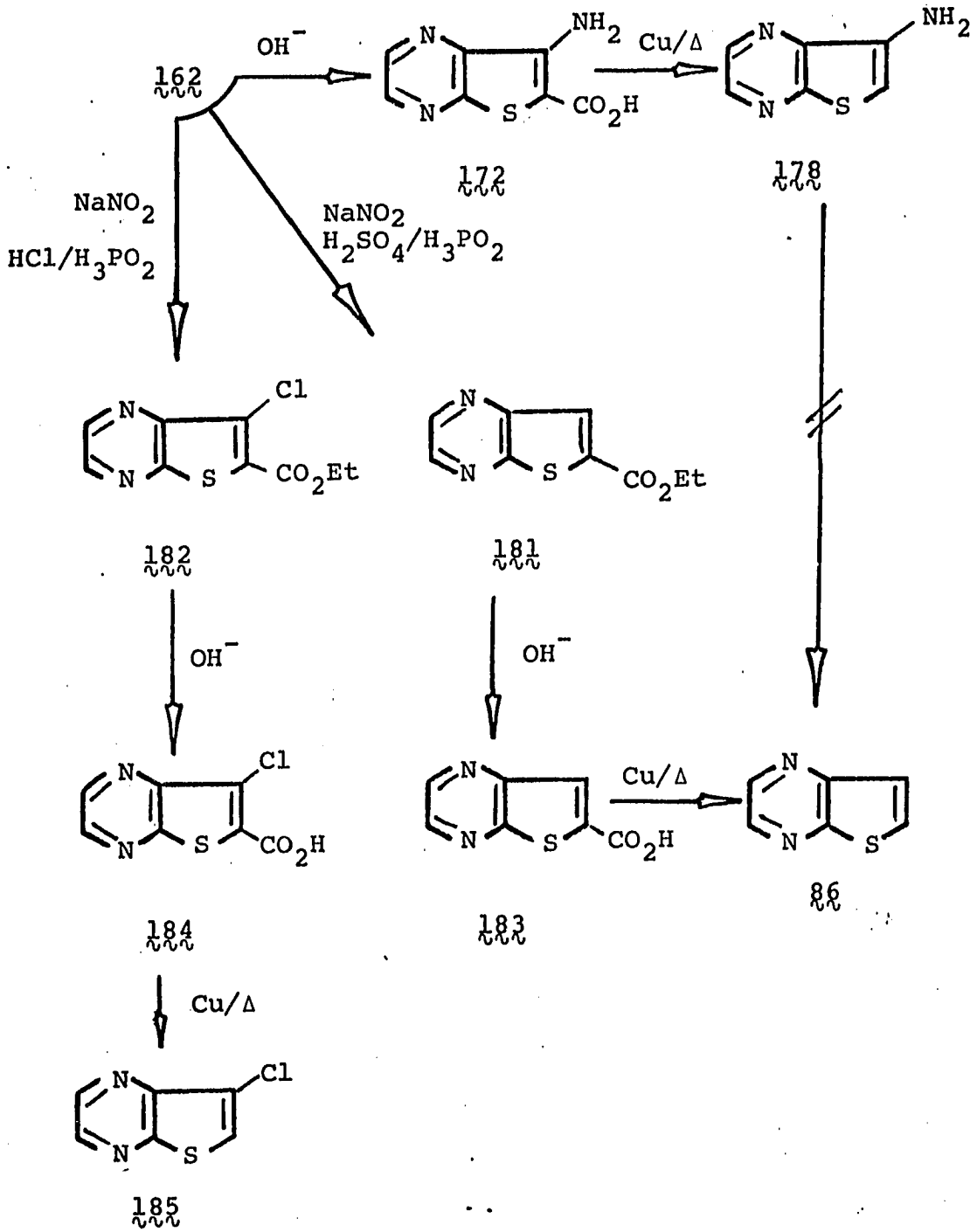
Pyrazino[2',3':4,5]thieno[3,2-d]-

pyrimidin-2,4-(1H,3H)-dione



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

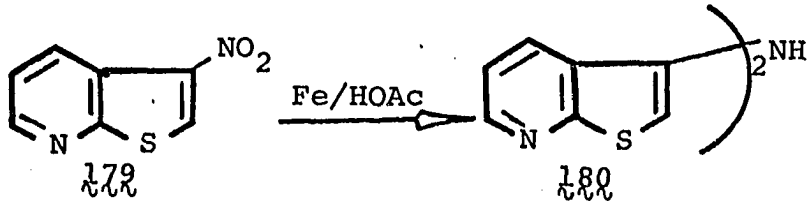
Chart XV
Synthesis of
Thieno[2,3-b]pyrazine



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

decarboxylation was attempted. Heating the sample of 172 in the absence of solvent resulted in a small amount of what appeared to be the desired product accompanied by large amounts of tars. The decarboxylation was attempted without quinoline by simply heating a dispersion of 172 and copper powder in diphenyl ether, but again only minimal conversion was realized. However, application of Paudler's recently disclosed copper powder procedure[122] to 172 resulted in an excellent yield of 7-aminothieno[2,3-b]-pyrazine (178, Chart XV). This procedure became the method of choice for this and all other decarboxylations attempted.

With compound 178 available there only remained a straightforward diazotization/reduction to realize the previously unknown thieno[2,3-b]pyrazine (86). Unfortunately, the diazotization of 178 did not produce 86 but instead led to a high melting product which was not characterized further. It should be mentioned, however, that this product was reminiscent of the results experienced by Klemm[123] when he reduced 3-nitrothieno[2,3-b]pyridine (179, below) in the presence of acid and recovered only the condensation product bis-(3-thieno[2,3-b]pyridyl)amine (180). This route was therefore abandoned.



179 180

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Initially the diazotization/reduction of 162 was equally as frustrating. When 162 (Chart XV) was diazotized in dilute hydrochloric acid followed by reduction with hypophosphorus acid a mixture of products resulted as was evident by the presence of two ester carbonyl absorption bands in the infrared spectrum of the product mixture. These products differed only moderately in their physical properties although it was possible to affect a partial separation via their somewhat different solubilities in ether. The PMR spectra of the partially separated products indicated that the lower melting product (73-74°) was the desired compound 181 because of the resonance at 8.08 δ (s, H-7), whereas the higher melting (104-105°) material showed no resonance that could be attributed to a proton at the C-7 position.

The diazotization of 162 in a more concentrated hydrochloric acid solution (26%), a modification which gave excellent results with ethyl 3-aminothieno[2,3-b]pyridine-2-carboxylate (116) affording ethyl thieno[2,3-b]pyridine-2-carboxylate (142), here also resulted in a single product. The PMR spectrum indicated that the product was not the desired compound (181). With the additional aid of the elemental analysis, it was possible to assign structure 182 to this product. This indicated that even the poorly nucleophilic chloride ion was able to replace the diazotized amine of 162 even at ice temperatures. From this observation it was concluded that a different acid was necessary for the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

diazotization, one possessing a counter ion with poorer nucleophilic characteristics than the chloride ion. The obvious solution was to attempt the diazotization using hypophosphorus acid (the subsequent reducing agent) as the proton source. This approach was dismissed when it was discovered that 162 did not form homogeneous solutions even when the hypophosphorus acid was used in large excess (0.5 g of 162 to 40 ml of hypophosphorus acid).

The next approach was to use sulfuric acid as the diazotizing solvent. Again, 162 was not soluble in dilute (20%) sulfuric acid, but it was found that by using 70% sulfuric acid complete homogeneity was obtained, resulting in blood-red solutions. This was quite satisfactory and the diazotization and subsequent reduction of the amine function of 162 could be accomplished to give 181 under these conditions. Two minor problems were encountered, however, which are note-worthy. First, the aqueous sodium nitrite had to be added very gradually since the introduction of water into this solvent system caused a very rapid rise in temperature. Secondly, it was possible only to maintain the reaction temperature in the range of 5-10° because of the fact that precipitation (presumably of the diazonium sulfate or frozen sulfuric acid) occurs when the temperature is at or near zero degrees.

In summary, depending on the acid used for the diazotization solvent, either 181 or 182 can be obtained from the diazotization of 162: if hydrochloric acid (26%)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

is used then 182 results and if sulfuric acid is employed then, following reduction, 181 is obtained.

Both of these products were separately saponified with alcoholic potassium hydroxide to give the corresponding 6-carboxylic acid derivatives 183 and 184, which were subsequently decarboxylated according to the procedure discussed above to give thieno[2,3-b]pyrazine (86) and 7-chlorothieno[2,3-b]pyrazine (185), respectively. This provided the desired, previously unreported parent heterocycle (86) and also a compound (185) of considerable synthetic utility. To elaborate briefly, compounds 181 and 183 possess functionality at C-6 which would enable synthetic manipulations specifically at this position; similarly, a halogen at the 7-position, as in 185, under controlled conditions[124] could undergo halogen-metal exchange with subsequent functionalization (*e.g.*, formylation) specifically at the C-7 position. This, of course, would ultimately provide synthetic avenues into thieno[2,3-b]-pyrazines with various fuctionalities at either the C-6 or C-7 position.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

EXPERIMENTAL

The primary achievement of science is the humility and honesty with which it constantly corrects its own errors. It is this that makes science the greatest of humanities.

-L.S. Kubie
Daedalus, 91, 305 (1962).

Melting Points. The temperatures are in degrees celsius and were determined in open capillary tubes using a Mel-Temp heated block apparatus and are not corrected.

Mass Spectra. The mass spectra were determined on a Varian MAT CH-7 instrument at Indiana University, Bloomington, Indiana.

Elemental Analyses. The microanalyses were performed by Galbraith Laboratories, Knoxville, Tennessee and Het-Chem-Co., Harrisonville, Missouri.

Infrared and PMR Spectra. The infrared spectra of compounds indicated by a † follow this section. A PMR spectrum appears there also for those compounds designated with a §.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

List of Experiments

Number	Compound	Page
Section I:		
1	ω-Nitro-2-vinylthiophene	97
2	ω-(2-Thienyl)ethylamine	97
3	Thieno[3,2-c]pyridine	98
4	2-Thienylpropenoic acid	99
5	4-Oxo-5H-thieno[3,2-c]pyridine	99
6	4-Chlorothieno[3,2-c]pyridine	100
7	Thieno[3,2-c]pyridine	100
8	Attempted Bromination of Thieno[3,2-c]-pyridine	101
9	Attempted Mannich Reaction on Thieno-[3,2-c]pyridine	101
10	Attempted Reactions Directed at Preparing the Ethyl Ester of 126	102
11	Attempted Reactions Directed at Preparing the Ethyl Ester of 124	104
12	3,4-Dimethoxybenzyl chloride	105
13	Reissert Compound of Thieno[3,2-c]-pyridine	105
14	4-(3',4'-Dimethoxybenzyl)thieno[3,2-c]-pyridine	106
15	Acidic Hydrolysis of the Reissert Compound 104	108
16	Preparation of Compound 113	109
17	4-Methylthiophene-2-carboxaldehyde	109

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Number	Compound	Page
18	3-(4-Methyl-2-thienyl)propenoic acid.....	110
19	3-Methyl-4-oxo-5H-thieno[3,2-c] pyridine.....	110
20	3-Methyl-4-chlorothieno[3,2-c]pyridine.....	111
21	3-Methylthieno[3,2-c]pyridine.....	112
22	Attempted Formation of 3-Cyanomethyl-thieno[3,2-c]pyridine.....	112
Section II:		
1	Nicotinamide-1-oxide.....	113
2	2-Chloronicotinonitrile.....	114
3	Ethyl 3-aminothieno[2,3-b]pyridine-2-carboxylate.....	114
4	Pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4(3H)-one.....	115
5	4-Chloropyrido[3',2':4,5]thieno[3,2-d]-pyrimidine.....	115
6	4-Methoxypyrido[3',2':4,5]thieno[3,2-d]-pyrimidine.....	115
7	4-Mercaptopyrido[3',2':4,5]thieno[3,2-d]-pyrimidine.....	116
8	4-Hydrazinopyrido[3',2':4,5]thieno[3,2-d]-pyrimidine.....	116
9	4-(N-Methylhydrazino)pyrido[3',2':4,5]-thieno[3,2-d]pyrimidine.....	117
10	4-(N-Morpholino)pyrido[3',2':4,5]thieno-[3,2-d]pyrimidine.....	117

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Number	Compound	Page
11	N,N'-Bis(pyrido[3',2':4,5]thieno-[3,2-d]pyrimidin-4-yl)piperazine.....	117
12	*s*-Triazolo[4,3-c]pyrido[3',2':4,5]thieno-[3,2-d]pyrimidine.....	117
13	Tetrazolo[1,5-c]pyrido[3',2':4,5]thieno-[3,2-d]pyrimidine.....	118
14	Pyrido[3',2':4,5]thieno[3,2-d]pyrimidine....	118
15	4-Methylthiopyrido[3',2':4,5]thieno-[3,2-d]pyrimidine.....	119
16	3-Methylpyrido[3',2':4,5]thieno[3,2-d]-pyrimidin-4(3H)-one.....	120
17	Ethyl Thieno[2,3-b]pyridine-2-carboxylate...	120
18	Thieno[2,3-b]pyridine-2-carboxylic acid.....	121
19	Thieno[2,3-b]pyridine.....	121
20	3-Aminothieno[2,3-b]pyridine-2-carboxamide..	122
21	Pyrido[3',2':4,5]thieno[3,2-d]-*v*-triazin-4(3H)-one	123
22	3-Aminothieno[2,3-b]pyridine-2-carbonitrile.	123
23	2,4-Diaminopyrido[3',2':4,5]thieno[3,2-d]pyrimidine.....	124
24	3-Amino-2-acetylthieno[2,3-b]pyridine.....	125
25	2-Benzylmercaptonicotinonitrile.....	125
26	3-Amino-2-phenylthieno[2,3-b]pyridine.....	125
27	Nicotinic acid 1-oxide.....	126
28	Ethyl Nicotinate 1-oxide.....	126
29	Ethyl 2-Chloronicotinate.....	127

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Number	Compound	Page
30	Ethyl 3-hydroxythieno[2,3-b]pyridine-2-carboxylate	127
31	3-Hydroxythieno[2,3-b]pyridine	127
Section III		
1	2-Hydroxypyrazine-3-carboxamide	128
2	2-Chloro-3-cyanopyrazine	129
3	Ethyl 7-Aminothieno[2,3-b]pyrazine-6-carboxylate	129
4	Ethyl 7-Amino-2,3-dimethylthieno[2,3-b]-pyrazine-2-carboxylate	130
5	7-Aminothieno[2,3-b]pyrazine-6-carboxylic acid	130
6	7-Aminothieno[2,3-b]pyrazine	131
7	Ethyl 7-Chlorothieno[2,3-b]pyrazine-6-carboxylate	131
8	7-Chlorothieno[2,3-b]pyrazine-6-carboxylic acid	132
9	7-Chlorothieno[2,3-b]pyrazine	132
10	Ethyl Thieno[2,3-b]pyrazine-6-carboxylate	132
11	Thieno[2,3-b]pyrazine-6-carboxylic acid	133
12	Thieno[2,3-b]pyrazine	134
13	Pyrazino[2',3':4,5]thieno[3,2-d]pyrimidin-4(3H)-one	134
14	4-Chloropyrazino[2',3':4,5]thieno[3,2-d]-pyrimidine	134
15	4-Methoxypyrazino[2',3':4,5]thieno[3,2-d]-pyrimidine	135

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Number	Compound	Page
16	4-Mercaptopyrazino[2',3':4,5]thieno[3,2-d]-pyrimidine.....	135
17	4-(N-Morpholino)pyrazino[2',3':4,5]thieno-[3,2-d]pyrimidine.....	135
18	N,N'-Bis(pyrazino[2',3':4,5]thieno[3,2-d]-pyrimidin-4-yl)piperazine.....	136
19	4-Hydrazinopyrazino[2',3':4,5]thieno-[3,2-d]pyrimidine.....	136
20	*s*-Triazolo[4,3-c]pyrazino[2',3':4,5]thieno-[3,2-d]pyrimidine.....	136
21	Pyrazino[2',3':4,5]thieno[3,2-d]pyrimidine..	137
22	2-Methyl-4H-pyrazino[2',3':4,5]thieno-[3,2-d][3,1]oxazin-4-one.....	138
23	2-Methylpyrazino[2',3':4,5]thieno[3,2-d]-pyrimidin-4(3H)-one.....	138
24	7-Aminothieno[2,3-b]pyrazine-6-carboxamide..	
25	7-Amino-6-cyanothieno[2,3-b]pyrazine.....	139
26	Pyrizino[2',3':4,5]thieno[3,2-d]pyrimidin-2,4(1H,3H)-dione.....	139

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

I. Synthesis of Thieno[3,2-c]pyridine and Derivatives

1. ω-Nitro-2-vinylthiophene.[81] A mixture of 33 ml (0.35 mol) of freshly distilled thiophene-2-carboxaldehyde and 20.0 g (0.32 mol) of nitromethane in 70 ml of methanol was cooled to -5° in a sodium chloride-ice-water bath and stirred mechanically while 14.0 g of sodium hydroxide dissolved in 50 ml of water was added through an addition funnel at such a rate that the temperature did not rise above 5°. The mixture was then stirred for an additional 1 hr in the sodium chloride-ice-water bath after which time it was diluted with 175 ml of cold water and then poured slowly, with swirling, into a cold solution of 70 ml of concentrated hydrochloric acid in 100 ml of water. This mixture was kept as cold as possible because the yellow crystals that are deposited rapidly turn green upon warming. The precipitate was filtered and washed with cold water and recrystallized from aqueous methanol to give 26.2 g (44%) of faint yellow crystals, mp 79-80°. Literature value 88-89°,[81] 80-81°.[125]

2. (2-Thienyl)ethylamine.[125] To a 1 l. three-necked round bottom flask, equipped with a condenser, nitrogen inlet and addition funnel, was added 500 ml of anhydrous diethyl ether and 20.0 g (0.129 mol) of lithium aluminum hydride. To this stirred suspension was added 23.0 g (0.148 mol) of ω-nitro-2-vinylthiophene at such a rate as to maintain gentle refluxing. After addition was complete, the mixture

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

was stirred for one hour and then the excess lithium aluminum hydride was carefully decomposed by the addition of water. The aluminum salts were filtered and washed with ether and then the combined ether filtrates were dried over anhydrous magnesium sulfate. After filtration and evaporation of the ether, the residue was distilled (51°, 1.5 mm) to give 12.0 g (73%). Literature[125] boiling point 45°, 1 mm.

3. Thieno[3,2-c]pyridine.[81] To 12.0 g (0.094 mol) of (2-thienyl)ethylamine was added 29.0 g of a 20% aqueous formaldehyde solution. Initially the reaction was exothermic. The solution was stirred for 3 hr in a boiling water bath and then for 1 hr at ambient temperature. The organic layer was separated, dissolved in benzene and washed three times with water. After drying over anhydrous magnesium sulfate, the benzene was removed on a rotovap to leave 10.2 g of the crude formimine of ω-(2-thienyl)ethylamine. This crude formimine was stirred for 2 hr with 15 ml of 20% hydrochloric acid and then the water was removed on a rotovap to give 11.7 g of crude (brownish-red) 4,5,6,7-tetrahydrothieno[3,2-c]pyridinium chloride. This crude salt was dissolved in 170 ml of a 50:50 mixture of water and dioxane to which was then added a solution of 164 g of potassium hydroxide in 210 ml of water and 110 g of potassium ferricyanide in 415 ml of water with rapid stirring. The mixture was then heated on a boiling water bath for 20 min and then allowed to cool to room temperature. On cooling a precipitate

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

formed and water was added in sufficient quantities to effect homogeneity. This mixture was then extracted with chloroform (5 X 100 ml) and then the combined chloroform extracts were washed with water and dried over anhydrous magnesium sulfate. After removal of the chloroform (rotovap), the residue was distilled (65°, 0.45 mm) and the distillate solidified upon cooling to give 1.8 g (20%), mp 41-42°. Literature value[81] 42-43°.

4. 2-Thienylpropenoic acid.[126] A mixture of 36.4 g (0.325 mol) of freshly distilled thiophene-2-carboxaldehyde and 50.7 g (0.487 mol) of malonic acid in 100 ml of pyridine and 2 ml of piperidine was refluxed for 2.5 hr. After cooling to ambient temperature, the solution was poured into 500 ml of ice and water and acidified until precipitation was complete. The product was filtered and air dried to give 36 g (72%), mp 140-143°. Literature value[126] 145°.

5. 4-Oxo-5H-thieno[3,2-c]pyridine.[82] To a solution of 92 ml (0.66 mol) of triethylamine in 1 l. of acetone was dissolved 100.0 g (0.65 mol) of 2-thienylpropenoic acid and this was then cooled in an ice-salt bath. To this solution was slowly added a solution of 51.5 ml (0.66 mol) of ethyl chloroformate in 50 ml of acetone. After stirring for 30 min 45.5 g (0.70 mol) of sodium azide in 100 ml of water was slowly added and stirring was continued for an additional 30 min in the ice-salt bath. The mixture was then poured over ice and water, filtered and the precipitate air dried to give the intermediate acyl azide (mp 75-80°

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

with vigorous effervescence, ir 4.68 μ). This crude intermediate was dissolved in 500 ml of dichloromethane and dried over anhydrous sodium sulfate. The dried dichloromethane solution was then slowly added to a preheated mixture of 330 ml of diphenyl ether and 100 ml of tri-n-butylamine at such a rate that the dichloromethane could be conviently collected. After the addition was completed, the mixture was heated for an additional 2 hr at about 230° and then cooled to room temperature. The precipitate was filtered and recrystallized from water to give 22.2 g (34%) of lightly tinted yellow crystals, mp 208-210°. Literature value[82] 213-214°.

6. 4-Chlorothieno[3,2-c]pyridine.[82] To 73 ml of phosphorus oxychloride was added 24.0 g (0.159 mol) of very dry 4-oxo-5H-thieno[3,2-c]pyridine. The heterogeneous mixture became homogeneous upon heating and the mixture was refluxed for 4.5 hr. After cooling, the excess phosphorus oxychloride was distilled (water aspirator) and the residue poured over ice. The precipitate which formed was filtered and air dried. (It should be noted that if the precipitate is allowed to stand for any length of time in the water, it will dissolve and subsequent work-up by extraction with chloroform will be necessary.) The dried precipitate weighed 26.6 g (98%) and was sufficiently pure for further reactions; mp 94-95°. Literature value[82] 96°.

7. Thieno[3,2-c]pyridine.[82] In a 1 l. round bottom flask fitted with a mechanical stirrer was added 26.6 g

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(0.157 mol) of 4-chlorothieno[3,2-c]pyridine, 100 g of zinc dust and 500 ml of glacial acetic acid. The mixture was refluxed for 12 hr, cooled, filtered and the filtrate concentrated to about 50 ml on a rotovap. The residue was made alkaline by the addition of 10% sodium hydroxide and then was extracted with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate, filtered, the chloroform evaporated and the residue was distilled (105-110°, 10 mm) to give on cooling 15.1 g (71%) of white crystals, mp 41-42°, identical in all respects to that made previously in Experiment Number 3 (p. 98).

8. Attempted Bromination of Thieno[3,2-c]pyridine.[81] To a solution of 1.8 g (14 mmol) of thieno[3,2-c]pyridine in 70 ml of thionyl chloride was added 0.77 ml (14 mmol) of bromine in 105 ml of thionyl chloride dropwise with stirring. After heating on a boiling water bath for 8 hr, the excess thionyl chloride was removed by distillation and the residue was added to ice water. The aqueous solution was neutralized by the addition of solid sodium bicarbonate and then extracted with ether. After drying over anhydrous sodium sulfate, the ether was evaporated and the residue chromatographed on a column of silica gel (3 cm X 30 cm) and eluted with a mixture of ether-petroleum ether (4:1). Two fractions separated, neither of which could be induced to crystalize.

9. Attempted Mannich Reaction on Thieno[3,2-c]pyridine In a 100 ml three-necked flask, fitted with a condenser and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

gas inlet was placed 30 ml of n-butyl alcohol. Hydrogen chloride gas was bubbled through the liquid until the weight of the flask and its contents had changed by 1.85 g (0.051 mol of hydrogen chloride). Anhydrous dimethylamine gas was then bubbled through this solution, an exothermic reaction ensued and after about 10 min the flask was cooled (the amine hydrochloride precipitated) and weighed. The change in weight was 2.05 g representing about 0.047 mol of dimethylamine hydrochloride. To this suspension was added 0.5 g (3.7 mmol) of thieno[3,2-c]pyridine and then 0.39 g of paraformaldehyde was added in portions. After the addition was complete, the mixture was refluxed for 0.5 hr, cooled, and the n-butyl alcohol was removed on a rotovap. The residue was dissolved in dilute hydrochloric acid, made alkaline by the addition of potassium carbonate and extracted with ether. The ether extracts were dried over anhydrous magnesium sulfate and the ether removed (rotovap) to leave a yellow oil. The picrate molecular charge transfer complexes of this product and the starting material were made and both melted at 230°. Distillation of the yellow oil resulted in > 90% recovery of starting material.

10. Attempted Reactions Directed at Preparing the Ethyl Ester of 126.

(a) 1 g (6.5 mmol) of 3-methyl-4-nitropyridine-1-oxide[136] in 15 ml of dimethylformamide was added dropwise to a mixture of 0.7 g (6.5 mmol) of ethyl chloroformate and 0.16 g (6.5 mmol) of sodium hydride in 20 ml of dimethylformamide. The

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

mixture was stirred at room temperature for 24 hr and then poured over water. Extraction of the aqueous solution resulted in recovery of starting material.

(b) A mixture of 5 g (32.5 mmol) of 3-methyl-4-nitropyridine-1-oxide, 1 ml of piperidine and 3.25 g of ethyl chloroformate in 25 ml of absolute ethanol was refluxed for 4 hr. After cooling the ethanol was removed to leave a viscous oil which solidified on cooling and was shown to be starting material.

(c) In an adaptation of a literature procedure,[113] 2.0 g (13 mmol) of 3-methyl-4-nitropyridine-1-oxide dissolved in 25 ml of benzene was added to a stirred solution of sodium diisopropylamide (prepared from 1.3 g (13 mmol) of diisoproylamine and 0.312 g of sodium hydride in 25 ml of benzene) and the mixture was stirred for 30 min. Then 1.4 g (23 mmol) of ethyl chloroformate in 10 ml of benzene was added and the mixture stirred at room temperature for 1 hr. The mixture was then poured over 400 ml of ice and water, the organic layer separated and the aqueous layer extracted with benzene. The combined organic layers were dried and the benzene removed to give starting material.

(d) To 1.63 g (25.9 mmol) of diisopropyl amine in 50 ml of dry benzene was added 1.1 g of a 57% dispersion of sodium hydride and the mixture was stirred in an ice bath for 30 min. Then 2 g (25.9 mmol) of 3-methyl-4-nitropyridine-oxide in 50 ml of benzene was added and stirred for another 30 min. Finally, 1.5 g (12.9 mmol) of diethyl carbonate in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

50 ml of benzene was added and the mixture was stirred for 1 hr, poured over ice and the organic layer separated. Extraction of the aqueous layer with chloroform followed by drying the chloroform extracts over anhydrous sodium sulfate and then evaporating the chloroform resulted in recovery of starting material

11. Attempted Reactions Directed at Preparing the Ethyl Ester of 124.

(a) To 2.2 g (0.022 mol) of diisopropylamine in 50 ml of benzene was added 0.92 g of sodium hydride and the mixture stirred in an ice water bath for 30 min. Then 3 g (0.022 mol) of 3-methyl-4-nitropyridine-1-oxide in 50 ml of benzene was slowly added and the mixture stirred for 30 min in an ice water bath. Then 2.56 g (0.022 mol) of diethyl carbonate was slowly added and the mixture stirred at room temperature for 30 min. The mixture was then poured over ice, acidified with concentrated hydrochloric acid and the layers separated. The aqueous layer was then neutralized by the addition of 10% sodium hydroxide and extracted with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate and the chloroform removed to leave an oil which was distilled and shown to be starting material.

(b) To a solution of sodium ethoxide in ethanol (prepared by dissolving 0.17 g of sodium in 40 ml of absolute ethanol) was added 1 g (7.25 mmol) of 3-methyl-4-nitropyridine.[137] The solution became orange and then 0.88 g (7.25 mmol) of diethyl carbonate was added and the mixture

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

stirred at room temperature for 0.5 hr. There was no appreciable change in color and therefore the mixture was refluxed for 2 hr. The reaction mixture became dark in color and a precipitate formed. The precipitate was filtered but did not melt below 300°. The ethanol was removed and water was added to the residue and the aqueous mixture was extracted with chloroform. Removal of the chloroform resulted in recovery of starting material.

12. 3,4-Dimethoxybenzyl chloride (veratryl chloride).[127] To a mixture of 5.15 g (30.6 mmol) of veratryl alcohol and 1 ml of pyridine in 75 ml of anhydrous ether was added 7.5 g of thionyl chloride dissolved in 40 ml of absolute ether in 10 ml portions. After the addition was complete, the reaction mixture was stirred at room temperature for 30 min. The light yellow solution was extracted with water (3 X 50 ml) and then dried over anhydrous magnesium sulfate. The ether was removed to leave an oil which solidified rapidly to a waxy solid when cooled and scratched to give 4.7 g (82.4%), mp 45-48°. Literature value[127] 48°.

13. Reissert Compound of Thieno[3,2-c]pyridine (104).† To a rapidly stirred mixture of 4.8 g (3.55 mmol) of 78 in 30 ml of dichloromethane and 6.19 g (0.106 mol) of potassium cyanide in 15 ml of water at room temperature was slowly added 9.87 g (0.071 mol) of benzoyl chloride. After the addition was completed, the mixture was stirred at room temperature overnight. The mixture was filtered and the layers separated. The aqueous layer was extracted with

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

dichloromethane (2 X 50 ml) and the combined organic extracts were washed successively with 5% hydrochloric acid, water, 5% sodium hydroxide, and water and then dried over anhydrous sodium sulfate. The dichloromethane was removed to give an oil which was triturated with petroleum ether and the crystalline product recrystallized from petroleum ether/ethanol to give white crystals, 1.2 g (11.2%), mp 119-121°. ir, see page 142.

Anal. Calcd. for $C_{15}H_{11}N_2ClOS$: C, 59.46; H, 3.66. Found: C, 59.51; H, 3.69.

14. 4-(3',4'-Dimethoxybenzyl)thieno[3,2-c]pyridine (112). Method A: To a stirred mixture of 2.1 g (6.9 mmol) of 104 and 1.98 g (10.6 mmol) of veratryl chloride in 75 ml of dimethylformamide cooled in an ice bath was added 0.98 g (40.8 mmol) of sodium hydride in two portions. As the sodium hydride was added, effervescence occurred and the solution became red. The mixture was stirred in an ice bath and slowly allowed to come to room temperature over a period of 4.5 hr. To this mixture was added 100 ml of water and spontaneous warming was noted along with a slight precipitation which eventually redissolved. The aqueous mixture was extracted with dichloromethane (6 X 50 ml) and the organic solvent was removed at the pump. To the residue was added 135 ml of 10% sodium hydroxide and 100 ml of 95% ethanol and the mixture was refluxed for 3 hr. The ethanol was removed (rotovap) and an oil separated. The aqueous solution was extracted with chloroform (5 X 100 ml) and then the chloro-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

form was extracted with 2N hydrochloric acid (5 X 100 ml). The hydrochloric acid extract was neutralized with solid sodium bicarbonate and then made strongly basic by the addition of 10% sodium hydroxide. The aqueous solution was re-extracted with chloroform (6 X 75 ml) to give a light yellow solution which was dried over anhydrous sodium sulfate and the chloroform romoved (rotovap) to leave an oil. Due to the quantity of oil it was assumed that some dimethylformamide had been carried through and therefore the excess dimethylformamide was distilled and the residue was triturated with petroleum ether to give white crystals which were recrystallized from 95% ethanol to give 0.9 g (45.8%), mp 94-95°. Literature value[105] 95-96°.

Method B: To 1.98 g (9.8 mmol) of tri-n-butylphosphine in 50 ml of dry benzene (distilled from sodium hydride) at room temperature, under a steady flow of nitrogen was added 1.83 g (9.8 mmol) of veratryl chloride in 20 ml of benzene. The mixture was refluxed for 18 hr, cooled to room temperature, and the benzene removed to give a viscous, jelly-like mass, which was suspended in 50 ml of dry (distilled from lithium aluminum hydride) dimethoxyethane in a round bottom flask equipped with a condenser and nitrogen gas inlet. This mixture was cooled to -35° in a controlled dry ice/acetone bath and to this mixture was slowly added 3.2 ml of 21% n-butyl lithium via a syringe through a rubber septum. The solution became yellow and was stirred at -35° for 0.5 hr after which time 0.83 g (4.88 mmol) of 4-chlorothieno[3,2-c]-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

pyridine in 20 ml of dry dimethoxyethane was slowly added. The reaction mixture was then allowed to come slowly to room temperature and then refluxed for 18 hr. To the reaction mixture was then added 1.06 g (0.01 mol) of sodium carbonate in 10 ml of water and the refluxing was continued for 3 hr. After cooling the reaction mixture was evaporated on a rotovap and the residue was taken up in 100 ml of ether. The ether solution was extracted with 5% hydrochloric acid (4 X 50 ml) and then this aqueous extract was made alkaline by the addition of 20% sodium hydroxide. The resulting suspension was extracted with ether; the ether extracts were combined and dried over anhydrous magnesium sulfate and then the ether was evaporated to leave an oil. Trituration of this oil with cold petroleum ether resulted in 0.5 g (36.4%) of white crystals, mp 93-95°, and identical in all respects with the previously reported product.[105]

15. Acidic Hydrolysis of the Reissert Compound 104

A mixture of 0.3 g (1.13 mmol) of 104 and 0.22 g (1.13 mmol) of 2,4-dinitrophenylhydrazine in 10 ml of concentrated hydrochloric acid was heated on a boiling water bath for 30 min. The reaction mixture was cooled and filtered to yield the 2,4-dinitrophenylhydrazone of benzaldehyde which was recrystallized from 95% ethanol, mp 234-236°. Literature value[128] 237°.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

16. Preparation of 113.[102] § A mixture of 350 mg (1.23 mmol) of 112 in 50 ml of absolute ethanol and 5 drops of 37% hydrochloric acid was hydrogenated over 100 mg of palladium on charcoal (5%) at atmospheric pressure until approximately 75 ml of hydrogen had been consumed. After filtering of the catalyst and washing it with warm ethanol, the ethanol was evaporated to leave an oil. To this crude oil was added 10 ml of water and enough 37% hydrochloric acid to achieve homogeneity. This was then heated on a steam bath and then 2 ml of 37% aqueous formaldehyde was added and heating continued for 1 hr. After cooling to room temperature, the solution was made basic with dilute sodium hydroxide and this solution was extracted with dichloromethane. The organic extracts were dried over anhydrous sodium sulfate and the solvent removed to leave an oil which crystallized very slowly. The PMR indicates (see page 156) that this is the desired compound. It was recrystallized from 95% ethanol with difficulty; mp 110°.

17. Preparation of 4-Methylthiophene-2-carboxaldehyde (97).[98] † In a 500 ml three-necked round bottom flask equipped with a condenser, rubber septum, and nitrogen inlet was placed 280 ml of anhydrous ether and 61.0 ml of 22% n-butyllithium (0.191 mol) at room temperature. To this solution was added 14.7 g (0.15 mol) of 3-methylthiophene in a slow but steady stream. The mixture was stirred at room temperature

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

for 2.5 hr and then added over a period of 45 min to a well stirred solution of 16.4 g (0.225 mol) of N,N-dimethylformamide in 60 ml of ether. The mixture was then stirred at ambient temperature for 36 hr and subsequently poured over ice and water and extensively extracted with ether. The combined ether extracts were dried over anhydrous magnesium sulfate and the ether evaporated to leave an oil which was distilled (93-95°, 2 mm) to give 14.3 g (75.6%) of a clear liquid which rapidly became light yellow. Literature value 84-86°, 1.8 mm.

18. 3-(4-methyl-2-thienyl)propenoic acid (98). A mixture of 14 g (0.113 mol) of 97 and 35.22 g (0.338 mol) of malonic acid in 75 ml of pyridine and 2 ml of piperidine was refluxed for 3.5 hr. After cooling the mixture was poured over ice and acidified with concentrated hydrochloric acid. The precipitate was collected and recrystallized from 95% ethanol to give 15.0 g (78.9%) of white crystals, mp 128-130°.

Anal. Calcd. for $C_8H_8O_2S$: C, 57.12; H, 4.79. Found: C, 57.26; H, 4.87.

19. 3-Methyl-4-oxo-5H-thieno[3,2-c]pyridine (99). To a solution of 9.0 g (0.089 mol) of triethylamine in 175 ml of acetone, cooled in an ice-water bath, was added 15 g (0.089 mol) of 98. To this stirred solution was slowly added 9.7 g (0.089 mol) of ethyl chloroformate in 30 ml of acetone. A precipitate formed and the reaction mixture was stirred for an additional 30 min. Then to this mixture was added 9.88 g (0.152 mol) of sodium azide dissolved in a minimum

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

of water. The resulting mixture was stirred for 30 min in the ice-water bath and then without the bath for 1.5 hr, after which time it was poured over ice and water and the precipitate filtered and air dried. This precipitate (*i.e.*, the acyl azide) was dissolved in 60 ml of dichloromethane and added slowly to a hot solution of 75 ml of diphenyl ether and 25 ml of tri-n-butylamine. The dichloromethane was distilled during the addition and when the last traces of dichloromethane were collected, the solution was heated just below boiling for 2 hours. Upon cooling to room temperature, a yellow precipitate formed which was filtered and recrystallized from benzene to yield 6.8 g (58.4 %), mp 195-197°(dec.).[†§]

Anal. Calcd. for $C_8H_7NOS \cdot \frac{1}{2}H_2O$: C, 55.70; H, 4.59. Found: C, 56.01; H, 4.55.

20. 3-Methyl-4-chlorothieno[3,2-c]pyridine (100)[§]. To 40 ml of phosphorus oxychloride was added 8.5 g (0.052 mol) of 99. Upon heating the solid dissolved and the mixture was refluxed for 4 hr. After cooling, the excess phosphorus oxychloride was distilled (water aspirator) and the residue poured over ice and water to give an initial white precipitate which dissolved as the solution warmed to room temperature. The aqueous solution was extracted with chloroform (5 X 100 ml), the combined extracts were dried over anhydrous sodium sulfate and the solvent removed on a rotovap to give a solid substance. This solid was sublimed (80°, 1.8 mm) to give 9.3 g (97%), mp 95-97°.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Anal. Calcd. for C_8H_6ClNS: C, 52.31; H, 3.29. Found: C, 52.22; H, 3.45.

21. 3-Methylthieno[3,2-c]pyridine (101)[§]. Into a 500 ml round bottom flask equipped with a mechanical stirrer was added 180 ml of glacial acetic acid, 36.0 g of zinc dust and 9.0 g (0.049 mol) of 100. This mixture was vigorously stirred and refluxed for 14 hr and then cooled to room temperature. The precipitate was filtered and washed with warm glacial acetic acid (2 X 50 ml) and then the filtrate was distilled (water aspirator) until the volume was about 50 ml. This solution was then made alkaline by the addition of 10% sodium hydroxide and then extracted with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate and then the solvent removed (rotovap) to leave an oil which was distilled (109-110°, 3 mm) to give 2.7 g (37%) of a clear liquid which slowly discolors unless kept in the refrigerator.

Anal. Calcd. for $C_8H_7NS \cdot \frac{1}{2}H_2O$: C, 60.75; H, 5.06. Found: C, 61.00; H, 5.21.

22. Attempted Formation of 3-Cyanomethylthieno[3,2-c] pyridine. To a gently refluxing solution of 0.5 g (3.4 mmol) of 101 and 0.03 g of benzoyl peroxide in 40 ml of carbon tetrachloride was added, in one portion, 0.6 g (3.5 mmol) of recrystallized N-bromosuccinimide. This solution was refluxed and irradiated with two 200 watt light bulbs for 2 hr. During this period a tarry residue appeared on the sides of the reaction vessel. After cooling slightly, the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

reaction mixture was decanted and the yellow solution allowed to cool to room temperature. It was then filtered and the carbon tetrachloride removed by distillation (water aspirator) under a stream of dry nitrogen to leave an oil which upon cooling solidified. This product was then dissolved in 12 ml of dimethylsulfoxide and added slowly to a cooled (18-20°) solution of 0.343 g (7.0 mmol) of sodium cyanide in 10 ml of dimethylsulfoxide and then reaction mixture allowed to stir in the bath for 1 hr and then overnight at room temperature. The reaction mixture was then poured over 100 ml of saturated aqueous sodium chloride solution and this was then extracted with ether. The ether was dried over sodium sulfate and then removed to leave an oil. The oil, which behaved as if it were hygroscopic, was dissolved in ether once again and dried over anhydrous magnesium sulfate and the resulting oil which occurred after the ether was removed was stoppered and refrigerated. Once again it seemed as if the product had absorbed water and therefore no further characterization was conducted at this time. A more complete investigation of this reaction is called for at a future date.

II. Synthetic Approaches to Thienopyridine Analogs of Antifolate Antimalarials

1. Nicotinamide-1-oxide. A mixture consisting of 15 g (0.123 mol) of nicotinamide, 24 ml of 30% aqueous hydrogen peroxide and 100 ml of glacial acetic acid was heated just

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

below its boiling point for 4 hr. After cooling the mixture was diluted with 300 ml of water and then evaporated (water aspirator) nearly to dryness. The white residue was dissolved in 150 ml of boiling water and filtered. Dilution of this solution with 40 ml of 95% ethanol and cooling in an ice bath resulted in the precipitation of white crystals which were filtered and air dried to yield 15.5 g (91%), mp 289-294°. Literature value[110] 291-293°.

2. 2-Chloronicotinonitrile. To 300 ml of phosphorus oxychloride was first added 32 g (0.232 mol) of nicotinamide-1-oxide and then 88 g of phosphorus pentachloride. The resultant mixture was refluxed for 4 hr, cooled to room temperature and the excess phosphorus oxychloride was distilled (water aspirator) and the residue poured over ice. The precipitate was collected and dried in a vacuum desicator over phosphorus pentoxide overnight to yield 14.5 g (44%), mp 104-105°. Literature value[110] 106-107°.

3. Ethyl 3-Aminothieno[2,3-b]pyridine-2-carboxylate (116).[107] † To a mixture of 3.4 g (24 mmol) of 2-chloro-3-cyanopyridine (*i.e.*, 2-chloronicotinonitrile (115)) and 2.65 g (25 mmol) of anhydrous sodium carbonate in 25 ml of absolute ethanol was added 3.0 g (25 mmol) of ethyl mercaptoacetate and the resulting mixture was refluxed for 6 hr with an accompanying color change of light brown to deep red. The reaction solution was cooled, evaporated to dryness on a rotovap, and the residue taken up in a small amount of water. The insoluble portion was filtered and the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

brownish yellow material recrystallized from boiling methanol as yellow crystals (23%), mp 181-182.5°.

Anal. Calcd. for $C_{10}H_{10}N_2O_2S$: C, 54.03; H, 4.53. Found: C, 54.15; H, 4.60.

4. Pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4(3H)-one (120)†. A solution of 2 g (9.0 mmol) of 116 in 25 ml of formamide was refluxed for 6.5 hr. Upon cooling a brown-green material precipitated which was filtered, dried and washed with warm ethyl acetate to leave a product which was recrystallized from 1-butanol/ethyl acetate as white crystals (89%), mp 340-342° (dec.).

Anal. Calcd. for $C_9H_5N_3OS$: C, 53.15; H, 2.47. Found: C, 52.95; H, 2.60.

5. 4-Chloropyrido[3',2':4,5]thieno[3,2-d]pyrimidine (129)†. To a mixture of 10 ml of pyridine and 20 ml of phosphorus oxychloride was added 2 g (9.9 mmol) of 120. The resulting solution was refluxed for 4 hr and after cooling was poured with vigorous stirring into ice water. The reddish-white crystals which formed after neutralization with sodium bicarbonate were filtered and recrystallized from 95% ethanol as colorless crystals (76%), mp 218-220°.

Anal. Calcd. for $C_9H_4ClN_3S$: C, 48.76; H, 1.82. Found: C, 48.61; H, 1.96.

6. 4-Methoxypyrido[3',2':4,5]thieno[3,2-d]pyrimidine (130). A solution of 1 g (4.54 mmol) of 129 in 5 ml of methanol and 20 ml of tetrahydrofuran containing 0.21 g (0.0098 g-atoms) of sodium was refluxed for 3 hr. Following

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

cooling of the solution, the methanol was removed on the rotovap and the residue treated with water. The aqueous solution was extracted with chloroform (4 X 25 ml) and the combined chloroform extracts were dried over anhydrous magnesium sulfate. Evaporation of the chloroform yielded a solid residue which upon recrystallization from hexane was obtained as yellow crystals (97%), mp 194-195°.

Anal. Calcd. for $C_{10}H_7N_3OS$: C, 55.28; H, 3.24. Found: C, 55.42; H, 3.31.

7. 4-Mercaptopyrido[3',2':4,5]thieno[3,2-d]pyrimidine (131). A solution of 2 g (9.0 mmol) of 129 and 1 g (13.0 mmol) of thiourea in 60 ml of 95% ethanol was refluxed for 2.5 hr. Upon cooling the product precipitated and was filtered and recrystallized from 95% ethanol as light yellow crystals (95%), mp 352-356°.

Anal. Calcd. for $C_9H_5N_3S_2$: C, 49.29; H, 2.30. Found C, 49.21; H, 2.45.

8. 4-Hydrazinopyrido[3',2':4,5]thieno[3,2-d]-pyrimidine (132)†. A solution consisting of 1 g (4.5 mmol) of 129 and 3 ml of 64% hydrazine hydrate in 30 ml of absolute ethanol was refluxed for 10 hr. Upon cooling the product precipitated and was filtered and recrystallized from absolute ethanol as white crystals (42%), mp 278-280°.

Anal. Calcd. for $C_9H_7N_5S$: C, 49.76; H, 3.25. Found C, 49.98; H, 3.28.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

General Procedure for 4-Substituted Aminopyrido[3',2':4,5]-thieno[3,2-d]pyrimidines (133, 137, 138).

An equimolar amount of the amine with 1 g (4.54 mmol) of 129 in 30 ml of absolute ethanol was refluxed for 5-48 hr. The solution was cooled and the product precipitated and was purified as indicated below.

9. 4-(N-Methylhydrazino)pyrido[3',2':4,5]thieno-[3,2-d]pyrimidine (133). This compound was obtained in 55% yield after refluxing for 48 hr and was recrystallized from 95% ethanol as white crystals, mp 279-280°.

Anal. Calcd. for $C_{10}H_9N_5S$: C, 51.93; H, 3.92. Found: C, 51.99; H, 4.10.

10. 4-(N-Morpholino)pyrido[3',2':4,5]thieno[3,2-d]-pyrimidine (137). This compound was obtained in 80% yield after refluxing for 10 hr and was recrystallized from ethanol as white crystals, mp 160-162°.

Anal. Calcd. for $C_{13}H_{12}N_4OS$: C, 57.34; H, 4.44. Found: C, 57.29; H, 4.47.

11. N,N'-Bis(pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine (138). This compound was obtained in 90% yield after 5 hr refluxing and was recrystallized from dimethylformamide as white crystals, mp >270° (dec.).

Anal. Calcd. for $C_{22}H_{16}N_8S_2$: C, 57.88; H, 3.53. Found: C, 57.98; H, 4.03.

12. s-Triazolo[4,3-c]pyrido[3',2':4,5]thieno[3,2-d]-pyrimidine (134).† A solution of 0.75 g (3.4 mmol) of 132 in 15 ml of 95% formic acid was refluxed for 2 hr. Upon

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

cooling the formic acid was removed *in vacuo*. The residue was dissolved in benzene/methanol (80:20) and filtered to remove a material which melted >370°. Evaporation of the benzene/methanol yielded a pale yellow material which was purified by sublimation *in vacuo* (220°, 1.5 mm) to yield a white product (46%), mp 228-232°.

Anal. Calcd. for $C_{10}H_5N_5S$: C, 52.85; H, 2.22. Found: C, 52.77; H, 2.30.

13. Tetrazolo[1,5-c]pyrido[3',2':4,5]thieno[3,2-d]-pyrimidine (135).†

Method A: To a suspension of 0.8 g (3.7 mmol) of 132 in 25 ml of 2N acetic acid at 45° was added 0.3 g (4.0 mmol) of sodium nitrite. Effervescence occurred immediately. After 1.5 hr the solution was cooled and the product filtered and recrystallized from aqueous acetone as cream crystals (47%), mp 214-216°.

Anal. Calcd. for $C_9H_4N_6S$: C, 47.36; H, 1.77. Found: C, 47.61; H, 1.84.

Method B: A solution of 1 g (4.54 mmol) of 129 and 0.6 g (9.0 mmol) of sodium azide in 25 ml of 95% ethanol was refluxed for 5 hr. Cooling yielded a cream colored product (40%) which upon recrystallization was identical to that described in Method A.

14. Pyrido[3',2':4,5]thieno[3,2-d]pyrimidine (136).†

Method A: Oxygen was bubbled through a 70 ml absolute ethanolic solution of 0.3 g of sodium (0.013 g-atom) and 0.4 g (1.85 mmol) of 132 for 1.5 hr. Following a multitude

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

of color changes and when the solution became deep purple, dilute hydrochloric acid was added until the solution became slightly acidic at which time sodium bicarbonate was carefully added to achieve neutrality. The resulting orange solution was extracted with ether and the ether extracts combined and dried over anhydrous sodium sulfate. Removal of the ether resulted in a residue which was recrystallized from ether to yield 0.28 g of white crystals (80%), mp 160-162°; mass spectrum m/e (relative intensity): 187(100,M^+), 160(36), 57(10).

Anal. Calcd. for $C_9H_5N_3S$: C, 57.73; H, 2.69. Found: C, 57.61; H, 2.80.

Method B: Evaporation of a 35 ml ethanolic solution of 0.6 g (2.72 mmol) of 129 and 50 mg of palladium on charcoal which had been treated with an atmospheric pressure of hydrogen for 2 days yielded a residue which following recrystallization from ether was identical in all respects to that described in Method A.

15. 4-Methylthiopyrido[3',2':4,5]thieno[3,2-d]-pyrimidine (139). To a solution of 0.15 g (0.0065 g-atom) of sodium in 100 ml of absolute ethanol was added 1 g (4.58 mmol) of 131, The solution was refluxed for 1 hr and then 2 ml of dimethyl sulfate was added slowly and the reflux resumed for an additional 4 hr. Upon cooling the solution was evaporated and the residue treated with water and the insoluble portion filtered, washed with water and recrystallized from hexane as white crystals (94%), mp

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

154-155°.

Anal. Calcd. for $C_{10}H_7N_3S_2$: C, 51.48; H, 3.02. Found: C, 51.51; H, 3.24.

16. 3-Methylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4(3H)-one (140).†

Method A: A solution of 2 g (9.0 mmol) of 116 in 25 ml of N-methylformamide was refluxed for 72 hr. Upon cooling to room temperature the product precipitated and was filtered and recrystallized from ethyl acetate as colorless crystals (54%), mp 230-232°.

Anal. Calcd. for $C_{10}H_7N_3OS \cdot \frac{1}{2}H_2O$: C, 53.08; H, 3.56. Found: C, 53.29; H, 3.63.

Method B: Methyl iodide (1 ml) was added dropwise with stirring to 0.2 g (1.0 mmol) of 128 dissolved in 2 ml of 1 M potassium hydroxide and 2 ml of dimethylformamide. After 10 min the product precipitated which, after filtering and recrystallization was identical to the material described in Method A.

17. Ethyl Thieno[2,3-b]pyridine-2-carboxylate (142)

In a mixture of 7 ml of 37% hydrochloric acid and 25 ml of water was dissolved 1.7 g (7.6 mmol) of 116 and the mixture was cooled in an ice-salt bath and stirred for 20 min. Then 0.5 g of sodium nitrite dissolved in 4 ml of water was added dropwise and then stirring in the ice-salt bath was continued for 30 min, at which time the solution was filtered and quickly added to a precooled 20 ml of hypophosphorus acid. Effervescence occurred and this solution was stirred in the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ice-salt bath for 1 hr and then placed in the refrigerator overnight. The solution was then filtered and the filtrate extracted with ether (6 X 50 ml). The combined ether extracts were dried over anhydrous magnesium sulfate and then the ether was removed on a rotovap without heat. The resulting thick orange oil crystallized upon standing all day in an ice bath, but when warmed to room temperature, the crystals again melted. The product was then distilled (Kugelrohr, 115-120°, 0.5 mm) to give light yellow crystals, 0.52 g (34%), mp 31-34°.

18. Thieno[2,3-b]pyridine-2-carboxylic acid (143)

To a suspension of 0.3 g of potassium hydroxide in 15 ml of absolute ethanol was added 0.6 g (2.9 mmol) of 142 and the mixture was refluxed for 1.5 hr. The heterogeneous mixture was cooled to room temperature and the solution filtered. The precipitate was dissolved in water (15 ml), filtered and acidified with glacial acetic acid and the resultant precipitate filtered, air dried and recrystallized from 95% ethanol with difficulty to yield 0.4 g (77%), mp 310-311°.

19. Thieno[2,3-b]pyridine (144). A mixture of 0.15 g (0.84 mmol) of 143 and 0,2 g (0.0031 g-atom) of cooper powder were mixed together in a 25 ml round bottom flask

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

equipped with a micro-distillation apparatus. The mixture was heated with an open flame, the mixture darkened and a liquid began to condense on the inside of the apparatus. After heating for about 3 min, the flame was removed and the reaction mixture allowed to cool. The entire apparatus was then extracted with ether and the combined ether extracts were washed with 10% sodium bicarbonate solution. After drying over anhydrous sodium sulfate, the ether was removed (rotovap) to leave an oil which crystallized when placed in the refrigerator. The PMR of this product and its melting point were identical with that reported for thieno[2,3-b]pyridine.[68] The yield was 0.1 g or 87%.

20. 3-Aminothieno[2,3-b]pyridine-2-carboxamide (155)†

Mercaptoacetamide was prepared in quantitative yield by bubbling ammonia gas through a solution of 24.0 g (0.20 mol) of ethyl mercaptoacetate dissolved in 35 ml of absolute ethanol at room temperature for 48 hr. After removal of the ethanol (rotovap) the residue solidified on cooling to give 18 g (98%), mp 148-152°. A mixture of 1.0 g (7.3 mmol) of 115, 0.7 g (7.3 mmol) of mercaptoacetamide and 2.31 g (0.22 mol) of sodium carbonate in 25 ml of absolute ethanol was refluxed for 6 hr. The volume of the reaction mixture was reduced (rotovap) to about 5 ml and then water was added to the residue. The pale green crystals which formed were filtered, air dried and recrystallized from aqueous methanol to give 0.7 g (50%) of off-white crystals, mp

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

249-250°.

Anal. Calcd. for $C_8H_7N_3OS$: C, 49.72; H, 3.65. Found: C, 49.99; H, 3.67.

21. Pyrido[3',2':4,5]thieno[3,2-d]-*v*-triazin-4(3H)one (156). Adapted from a literature procedure,[89] 0.8 g (4.1 mmol) of 155 was dissolved in 30 ml of concentrated sulfuric acid and then cooled in an ice bath. To this red solution was then added 0.31 g (4.5 mmol) of sodium nitrite in small portions. The mixture was then stirred at room temperature for 2 hr and then in a boiling water bath for 1 hr. After cooling, the reaction mixture was poured over ice and the aqueous mixture was extracted with ether (4 X 100 ml). The ether extracts were then extracted with 0.25 N sodium hydroxide (total volume 350 ml). The basic extracts were made slightly acidic by the addition of dilute hydrochloric acid and then this mixture was extracted once again with ether (3 X 100 ml). After drying over anhydrous magnesium sulfate, the ether was removed (rotovap) to leave a light yellow solid which was recrystallized from a large volume of 95% ethanol to give 0.2 g (25%),[130] mp 190-192°.

Anal. Calcd. for $C_8H_4N_4OS$: C, 47.05; H, 1.97. Found: C,

22. 3-Aminothieno[2,3-b]pyridine-2-carbonitrile (157).† In 15 ml of benzene (distilled from sodium) was placed 0.2 g (1.0 mmol) of 155 and 0.14 g of phosphorus pentoxide and the mixture was refluxed for 2.5 hr. After cooling, the benzene was decanted and the residue was carefully poured

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

over ice and water. The aqueous solution was then neutralized with solid sodium bicarbonate and then extracted with ether. The combined ether extracts were dried over anhydrous sodium sulfate and the ether evaporated to give a solid which was sublimed (155°, 2mm) to give 0.15 g (86%), mp 170-175°.

Anal. Calcd. for $C_8H_5N_3S$: C, 54.78; H, 2.87. Found: C, 54.61; H, 3.08.

23. 2,4-diaminopyrido[3',2':4,5]thieno[3,2-d]pyrimidine (158)[†]. A mixture of 0.25 g (1.75 mmol) of 157 and 0.22 g (1.9 mmol) of chloroformamidine hydrochlroide in 5 ml of diglyme (distilled from lithium aluminum hydride) was slowly heated to 150° in an oil bath. Initially the mixture was heterogeneous and finely divided but at about 140° the reactant clumped together. The mixture was heated at 150° for 1 hr, cooled and the solvent decanted. The residue was washed with ether, dissolved in boiling ethanol and treated with decolorizing charcoal. After filtering, the solution was cooled and the filtrate made basic with ammonium hydroxide and placed in the refrigerator. No crystals precipitated even after 4 days in the refrigerator. The solution was then made very basic by the addition of dilute sodium hydroxide and extracted with ether. The ether extracts were dried over anhydrous sodium sulfate and the ether removed to give a light yellow solid, 0.2 g (53%), mp > 300°.

Anal. Calcd. for $C_9H_7N_5S \cdot \frac{1}{2}H_2O$: C, 47.78; H, 3.53. Found: C, 48.10; H, 3.65.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

24. 3-Amino-2-acetylthieno[2,3-b]pyridine (145). †

To 25 ml of absolute ethanol was added 1.5 g (16.6 mmol) of mercaptoacetone,[131] 2.3 g (16.5 mmol) of 115 and 1.8 g (17.0 mmol) of anhydrous sodium carbonate and the mixture was refluxed for 6 hr. After cooling, the reaction mixture was treated with water to give a yellow precipitate. Condensation of the filtrate gave a second crop. The combined precipitates were recrystallized from aqueous ethanol to give 2.7 g (84.7%) of light yellow crystals, mp 196-198°.

Anal. Calcd. for $C_9H_8N_2OS$: C, 56.23; H, 4.19. Found: C, 56.05; H, 3.91.

25. 2-Benzylmercaptonicotinonitrile (146). † A mixture of 3.0 g (21.8 mmol) of 115, 2.35 g (22.0 mmol) of anhydrous sodium carbonate and 2.7 g (21.8 mmol) of benzylmercaptan in 35 ml of absolute ethanol was refluxed for 20 hr. The volume of the reaction mixture was then condensed to about 10 ml, water was added and the precipitate which formed was filtered and recrystallized from aqueous ethanol to yield 2.6 g (53%), mp 66-68°.

Anal. Calcd. for $C_{13}H_{10}N_2S$: C, 68.99; H, 4.45. Found: C, 69.03; H, 4.59.

26. 3-Amino-2-phenylthieno[2,3-b]pyridine (147). In a solution of sodium ethoxide and ethanol (prepared by dissolving 0.1 g of sodium (0.004 g-atom) in 20 ml of absolute ethanol) was place 0.2 g (0.88 mmol) of 146 and the mixture refluxed for 4 hr. To the reaction mixture which had become light yellow was added 20 ml of water and the solution was

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

extracted with chloroform (3 X 25 ml). The combined extracts were dried over magnesium sulfate and the solvent removed to leave an oil which solidified upon cooling. The solid was recrystallized from 95% ethanol and then sublimed (130°, 12 mm) to give 0.18 g (90%), mp 117-118°.

Anal. Calcd. for $C_{13}H_{10}N_2S$: C, 68.99; H, 4.45. Found: C, 69.00; H, 4.51.

27. Nicotinic acid 1-oxide (150). A mixture of 20 g (0.163 mol) of 149 and 45 ml of 30% hydrogen peroxide in 100 ml of glacial acetic acid was refluxed for 3 hr. After cooling the product was filtered and recrystallized from a very large volume of methanol to give 12.3 g (55%), mp 250-254° (dec.). Literature value[117] 249° (dec.).

28. Ethyl Nicotinate 1-oxide (151). Anhydrous hydrogen chloride gas was bubbled through a well stirred suspension of 12.3 g (0.088 mol) of 150 in 250 ml of absolute ethanol. After about 10 min the solution became homogeneous and heated up appreciably. After 1 hr the gas was disconnected and the reaction mixture was refluxed for 4 hr. After cooling the volume was reduced on a rotovap to about 50 ml and then this residue was taken up in 100 ml of dichloromethane and washed thoroughly with 10% sodium bicarbonate. After drying over anhydrous sodium sulfate, the solvent was removed to give 16 g (60%) of white crystals, mp 94-96°. Literature value[117] 97°.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

29. Ethyl 2-chloronicotinate (152). A mixture of 5 g (0.0299 mol) of 151 6.23 g (0.0299 mol) of phosphorus pentachloride in 30 ml of phosphorus oxychloride was refluxed for 3 hr. The excess phosphorus oxychloride was distilled (water aspirator) and the residue poured over ice. The product was an oil which settled on the bottom. The layers were separated and the aqueous layer extracted with chloroform. The extracts were dried over anhydrous sodium sulfate and the chloroform removed and the residue distilled (115°, 6.5 mm) to give 2.3 g (42%).

30. Ethyl 3-Hydroxylthieno[2,3-b]pyridine-2-carboxylate (153).†§ A mixture of 2.3 g (12.4 mmol) of 152, 1.56 g (13.0 mmol) of ethyl mercaptoacetate and 1.38 g (13.0 mmol) of sodium carbonate in 50 ml of absolute ethanol was refluxed for 5 hr. After cooling, the ethanol was removed and water added to the residue. The yellow crystals which formed were filtered and air dried. Recrystallization of aqueous ethanol afforded 1.5 g (54%), mp 255-258° (dec.).

31. 3-Hydroxythieno[2,3-b]pyridine (154)†. A solution of 0.25 g (1.12 mmol) of 153 in 20 ml of 70% sulfuric acid was refluxed for 1.5 hr, cooled to room temperature and poured over ice. The aqueous solution was neutralized with sodium bicarbonate and extracted with ether; the extracts dried and solvent removed to leave a brown oil which solidified on cooling. The crude mp was 154-160°. The spectral properties indicated

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

that the desired product was present and correlated well with those of the product obtained when the potassium salt of 3-aminothieno[2,3-b]pyridine-2-carboxylic acid was refluxed in aqueous acetic acid to give, according to analogous literature[116] preparations, the identical product, mp 169-173°.

III. Synthetic Approaches to Thieno-separated Analogs of Folic Acid

1. 2-Hydroxypyrazine-3-carboxamide (160). In a 500 ml round bottom flask was placed 25 g (0.173 mol) of 40% glyoxal and it was then diluted with 110 ml of water. To this solution was then added 36 g (0.346 mol) of sodium bisulfite which was mixed in thoroughly, resulting in an initial homogeneous solution from which a precipitate soon separated. Then 20 g (0.171 mol) of 2-aminomalonamide was added with vigorous stirring and the heterogeneous mixture was heated for 3 hr at 85°. After cooling to ambient temperature, 93 g (0.68 mol) of sodium acetate trihydrate was added with stirring so that all of the sodium acetate dissolved. To this mixture was then slowly added 45 ml of 30% hydrogen peroxide; the temperature spontaneously rose to 50° and was maintained at this temperature with intermittent cooling. After 1 hr the temperature began to decrease and the mixture was allowed to stir until the temperature had fallen to 35° and then the reaction mixture was placed in a refrigerator for 24 hr. Filtration of the reaction mixture afforded a light tan product which was boiled in water, cooled and filtered.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

After air drying, followed by drying in a vacuum desicator for 24 hr over phosphorus pentoxide, 12.6 g (53%) of the desired product was obtained; mp 264-266° (dec.). Literature value[120] 265-266°.

2. 2-Chloro-3-cyanopyrazine (161).[121] To 100 ml of phosphorus oxychloride was slowly added 16.5 g (0.12 mol) of 160 and the mixture was refluxed for 2.5 hr. After the excess phosphorus oxychloride was removed (water aspirator) by distillation, the residue was poured over ice and water and this aqueous deep red solution was extensively extracted with ether. Drying of the ether extracts over anhydrous magnesium sulfate and evaporation of the ether resulting in the crude product which was purified by vacuum distillation (95-97°, 3 mm) to give 7.5 g (44.3%) of a clear liquid which solidified upon cooling.

3. Ethyl 7-Aminothieno[2,3-b]pyrazine-6-carboxylate (162).[129] †§ To 7.5 g (53.7 mmol) of 161 in 150 ml of absolute ethanol was added 6.48 g (54.0 mmol) of ethyl mercaptoacetate, and 5.73 g (54.0 mmol) of anhydrous sodium carbonate and the mixture was refluxed for 4.5 hr. After cooling, the reaction mixture was filtered. The residue was stirred with 200 ml of water, filtered and the insoluble material recrystallized from aqueous ethanol to give 9.3 g (77.6%), mp 114-116°; mass spectrum m/e (relative abundance): 223 (69, M^+), 195 (20), 178 (23), 177 (100), 151 (11), 150 (14), 149 (32), 123 (14), 122 (13), 72 (15), 52 (10), 45 (13), 29 (15), 18 (21).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Anal. Calcd. for $C_9H_9N_3O_2S$: C, 48.41; H, 4.06. Found: C, 48.23; H, 4.14.

4. Ethyl 7-amino-2,3-dimethylthieno[2,3-b]pyrazine-6-carboxylate (163)†. A mixture of 1 g (6.0 mmol) of 2-chloro-3-cyano-5,6-dimethylpyrazine,[132] 0.65 g (6.2 mmol) of anhydrous sodium carbonate and 0.75 g (6.2 mmol) of ethyl mercaptoacetate in 20 ml of absolute ethanol was refluxed for 6 hr. After work-up as cited above, the crude product was recrystalized from 95% ethanol to give 1.2 g (80%) of light yellow crystals, mp 137-138°.

Anal. Calcd. for $C_{11}H_{13}N_3O_2S$: C, 52.57; H, 5.21. Found: C, 52.79; H, 5,23.

5. 7-Aminothieno[2,3-b]pyrazine-6-carboxylic acid (172)†. A mixture of 2.0 g (8.96 mmol) of 162 and 1.2 g (21.4 mmol) of potassium hydroxide in 50 ml of absolute ethanol was refluxed for 1 hr. After cooling the precipitate was collected, dissolved in water and then acetic acid was added until precipitation was complete. The product was filtered and air dried to yield 1.2 g (68.6%) of light yellow crystals, mp 218-220°; mass spectrum (relative abundance): 195 (100, M^+), 178 (10), 177 (100), 149 (45), 123 (11), 122 (30), 105 (11), 72 (15), 52 (21), 45 (40), 28 (20), 18 (12).

Anal. Calcd. for $C_7H_5N_3O_2S$: C, 43.07; H, 2.56. Found: C, 43.19; H, 2.69.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

6. 7-Aminothieno[2,3-b]pyrazine (178).†§ A mixture of 1 g (5.13 mmol) of 172 and 1.21 g (0.019 g-atom) of copper powder in a 50 ml round bottom flask fitted for distillation was heated with an open flame for about 3 min. Liquidification occurred and gas was vigorously evolved. After cooling, the entire apparatus was extracted with ether and then the combined ether extracts were washed with 10% sodium bicarbonate (2 X 20 ml), dried over anhydrous sodium carbonate and then the ether removed to give light yellow crystals which were sublimed (75°, 3 mm) to give the pure product, 0.5 g (65%), mp 81-82°.

Anal. Calcd. for $C_6H_5N_3S$: C, 47.66; H, 3.33. Found: C, 47.40; H, 3.28.

7. Ethyl 7-Chloro[2,3-b]pyrazine-6-carboxylate (182).†§ To a mixture of 20 ml of water and 60 ml of concentrated hydrochloric acid was added 1.12 g (5.0 mmol) of 162 which dissolve to give a blood red solution. This solution was cooled in an ice-salt bath to -3° and stirred at that temperature for 30 min. To this solution was added a solution of 0.5 g (7.0 mmol) of sodium nitrite dissolved in 4 ml of water dropwise so that the temperature remained at -3°. The solution underwent a color change to light orange. After stirring at -3° for an additional 45 min, this solution was added, in one portion, to 20 ml of pre-cooled 50% hypophosphorus acid and then this mixture was stirred at -3° for 30 min. The light yellow solution was then placed in a refrigerator for 18 hr. After filtering off a small

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

amount of insoluble material, the aqueous solution was extracted with chloroform (3 X 75 ml), the extracts dried over anhydrous magnesium sulfate and the solvent removed to give light yellow crystals which were recrystallized from 95% ethanol to give 0.8 g (77%) of white crystals, mp 104-105°.

Anal. Calcd. for $C_9H_7ClN_2O_2S \cdot H_2O$: C, 41.69; H, 3.47. Found: C, 41.67; H, 3.51.

8. 7-Chlorothieno[2,3-b]pyrazine-6-carboxylic acid (184). A mixture of 0.8 g (3.85 mmol) of 182 and 0.73 g of potassium hydroxide in 30 ml of absolute ethanol was refluxed for 1.5 hr. The ethanol was removed on a rotovap, water was added to the residue and the aqueous solution was acidified with glacial acetic acid to give a white precipitate which was filtered, air dried, and recrystallized from aqueous ethanol to give 0.3 g (44%), mp 248-250°.

Anal. Calcd. for $C_7H_4ClN_2O_2S$: C, 39.16; H, 1.39. Found: C, ; H, .

9. 7-Chlorothieno[2,3-b]pyrazine (185).§ A mixture of 0.25 g (1.39 mmol) of 184 and 0.329 g (0.0052 g-atom) of copper powder were reacted as described previously. After the usual work-up, the white solid that was obtained was sublimed (50°, 2 mm) to give 0.19 (100%), mp 70-72°.

Anal. Calcd. for $C_6H_3ClN_2S$: C, 42.23; H, 1.77. Found: C, 42.00; H, 1.62.

10. Ethyl Thieno[2,3-b]pyrazine-6-carboxylate (183).†§ To 50 ml of 70% sulfuric acid was added 1.0 g (4.48 mmol)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

of 162 and the deep red solution was cooled to 3-5° in an ice water bath. To this cold solution was added 0.5 g of sodium nitrite dissolved in 5 ml of water at such a rate that the temperature did not rise above 5°. This reaction solution was stirred for 45 min and then poured rapidly into 40 ml of pre-cooled 50% hypophosphorus acid. Vigorous foaming occurred and the stirring was continued in the ice water bath for 30 min and then the reaction mixture was placed in a refrigerator for 18 hr. The solution had darkened and a precipitate had formed. The precipitate was filtered and the filtrate extracted with chloroform (3 X 50 ml). The combined chloroform extracts were dried over anhydrous sodium sulfate and the chloroform evaporated to leave a solid which was combined with the initial precipitate. The crude product was recrystallized from 95% ethanol to give 0.6 g (64%) of a white product, mp 73-74.5°.

Anal. Calcd. for $C_9H_8N_2O_2S$: C, 51.91; H, 3.87. Found: C, 52.09; H, 3.97.

11. Thieno[2,3-b]pyrazine-6-carboxylic acid (183).[§]

A mixture of 0.22 g (1.06 mmol) of 181 and 0.2 g of potassium hydroxide in 25 ml of absolute ethanol was refluxed for 1 hr. After cooling to room temperature, the ethanol was removed (rotovap) and the residue dissolved in water, filtered, and acidified with 5% hydrochloric acid. The white precipitate which formed was collected and air dried and recrystallized from 95% ethanol to give 0.18 g (95%), mp 261-262 (dec.).

Anal. Calcd. for $C_7H_4N_2O_2S \cdot H_2O$: C, 42.42; H, 3.03. Found: C, 42.48; H, 3.09.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

12. Thieno[2,3-b]pyrazine (86).[†,§] A mixture of 0.23 g (1.28 mmol) of 183 and 0.32 g of copper powder were mixed and heated as described previously. After extraction with ether and washing the ether extracts with 10% sodium bicarbonate, the ether was dried over anhydrous magnesium sulfate and then removed to give an oil which crystallized upon cooling (with scratching). This product was sublimed (35-40°, 3 mm) to give 0.13 g (80%) of white crystals, mp 43-44.5°.

Anal. Calcd. for $C_6H_4N_2S$: C, 52.92; H, 2.96. Found: C, 52.90; H, 3.16.

13. Pyrazino[2',3':4,5]thieno[3,2-d]pyrimido-4(3H)-one (164).[†] A mixture of 5.0 g (22.4 mmol) of 162 in 150 ml of formamide was refluxed for 8 hr Upon cooling a light yellow precipitate resulted which was filtered and recrystallized from 1-butanol to yield 2.8 g (61%) of off white crystals, mp > 390°.

Anal. Calcd. for $C_8H_4N_4OS$: C, 47.05; H, 1.97. Found: C, 46.99; H, 2.27.

14. 4-Chloropyrazino[2',3':4,5]thieno[3,2-d]pyrimidine (165).[†] To 10 ml of phosphorus oxychloride was added 0.5 g (2.45 mmol) of 164 and the resulting mixture was refluxed for 3.5 hr. After cooling the reaction mixture was poured with vigorous stirring over ice. The tan crystals that resulted were filtered, dried, and then sublimed (150°, 1.5 mm) to give 0.38 g (70%) of white crystals, mp 175-177°; mass spectrum (relative abundance): 224 (37), 223 (10), 222 (100), 187 (85), 160 (17).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Anal. Calcd. for $C_8H_3ClN_4S$: C, 43.05; H, 1.34. Found: C, 43.01; H, 1.14.

15. 4-Methoxypyrazino[2',3':4,5]thieno[3,2-d]-pyrimidine (166). A solution containing 0.3 g (1.34 mmol) of 165 and 0.5 g of sodium in 15 ml of absolute methanol was refluxed for 3 hr. The solution was cooled, evaporated to dryness and the resulting residue treated with water. The insoluble material was filtered, air dried and then sublimed (140°, 1.5 mm) to give 0.1 g (34%) of white crystals, mp 216-218°.

Anal. Calcd. for $C_9H_6N_4OS$: C, 49.54; H, 2.75. Found: C, 49.61; H, 2.86.

16. 4-Mercaptopyrazino[2',3':4,5]thieno[3,2-d]-pyrimidine (167). A solution of 0.7 g (3.15 mmol) of 165 and 0.6 g (8.0 mmol) of thiourea in 30 ml of absolute ethanol was refluxed for 3 hr. After cooling, the yellow-orange mixture was filtered and the precipitate was recrystallized from a large volume of methanol to yield 0.66 g (94%) of yellow crystals, mp 365-367° (dec.); mass spectrum (relative abundance): 220 (100, M^+), 193 (22), 187 (28), 28(13), 18 (29).

Anal. Calcd. for $C_8H_4N_4S_2 \cdot \frac{1}{2}H_2O$: C, 42.10; H, 2.19. Found: C, 42.02; H, 2.17.

17. 4-(N-Morpholino)pyrazino[2',3':4,5]thieno[3,2-d]-pyrimidine (169). A solution of 0.1 g (0.45 mmol) of 165 and 1 ml of morpholine in 7 ml of absolute ethanol was refluxed for 10 hr. The solution was cooled, the ethanol evaporated

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

and the product obtained by filtering the residue. The collected material was washed with water, air dried and purified by sublimation (150°, 1.5 mm) to give 0.06 g (50%) of white crystals, mp 206-208°.

Anal. Calcd. for $C_{12}H_{11}N_5OS$: C, 52.75; H, 4.03. Found: C, 52.68; H, 4.24.

18. N,N'-Bis(pyrazino[2',3':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine (168). To 5 ml of absolute ethanol was added 0.1 g (0.45 mmol) of 165 and 0.2 g (0.23 mmol) of piperazine and the mixture was refluxed for 10 hr. Upon cooling the precipitate was collected and recrystallized from dimethylformamide to yield 0.1 g (48%), mp 336° (dec.).

Anal. Calcd. for $C_{20}H_{14}N_{10}S_2 \cdot 2H_2O$: C, 48.58; H, 3.62. Found: C, 48.21; H, 3.92.

19. 4-Hydrazinopyrazino[2',3':4,5]thieno[3,2-d]-pyrimidine (170).† To 0.5 g (2.25 mmol) of 165 in 20 ml of absolute ethanol was added 2 ml of 97% hydrazine hydrate and the mixture was refluxed for 12 hr. After cooling, the orange mixture was filtered and the precipitate recrystallized from absolute ethanol to give 0.45 g (91.7%), mp 256-258°; mass spectrum m/e (relative abundance): 218 (100, M^+), 188 (17), 161 (26).

Anal. Calcd. for $C_8H_6N_6S$: C, 44.02; H, 2.77. Found: C, 44.23; H, 2.91.

20. *s*-Triazolo[4,3-c]pyrazino[2',3':4,5]thieno[3,2-d]-pyrimidine (171).† A solution of 0.2 g (0.92 mmol) of 170 and 15 ml of formic acid was refluxed for 4 hr. After

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

cooling the formic acid was removed *in vacuo*. The residue was sublimed (220°, 1.5 mm) to yield 0.08 g (37%) of a white product, mp 274-276°, which appeared to be hygroscopic when exposed to air.

Anal. Calcd. for $C_9H_4N_6S \cdot \frac{1}{2}H_2O$: C, 45.56; H, 2.09. Found: C, 45.81; H, 2.12.

21. Pyrazino[2',3':4,5]thieno[3,2-d]pyrimidine (159).[§]

Method A: Oxygen was bubbled through a 40 ml absolute ethanolic solution of 0.2 g (0.0086 g-atom) of sodium and 0.2 g (0.917 mmol) of 170 for 3 hr. Dilute hydrochloric acid was then added to the solution until it was slightly acidic and then sodium bicarbonate was carefully added to achieve neutrality. The resulting solution was extracted with ether (4 X 40 ml) and the ether extracts were combined and dried over anhydrous sodium sulfate. The ether was removed to leave a green-brown residue which was sublimed (160°, 1.5 mm) to give 0.14 g (81.4%) of white crystals, mp 192-195°.

Anal. Calcd. for $C_8H_4N_4S$: C, 51.05; H, 2.14. Found: C, 50.99; H, 2.33.

Method B: A 35 ml ethanolic solution of 0.1 g (0.45 mmol) of 165 containing 50 mg of palladium on charcoal and 60 mg of magnesium oxide was treated with an atmospheric pressure of hydrogen for 4 days. Following this exposure, the solution was filtered, the catalyst washed with warm ethanol and the ethanolic filtrate evaporated. The residue was treated with water and extracted with ether (2 X 25 ml), the ether extracts combined and dried over anhydrous sodium sulfate and evaporated

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

to yield, after purification, 0.5 g (60%) of white crystals identical to that described in Method A.

22. 2-Methyl-4H-pyrazino[2',3':4,5]thieno[3,2-d]-[3,1]oxazin-4-one (173).† A solution of 0.7 g (3.68 mmol) of 172 in 15 ml of acetic anhydride was refluxed for 3 hr. After cooling to room temperature a white precipitate had formed, which was filtered and recrystallized from absolute ethanol to yield 0.5 g (64%) as white crystals, mp 198-202°.

Anal. Calcd. for $C_9H_5N_3O_2S$: C, 49.31; H, 2.29. Found: C, 49.55; H, 2.46.

23. 2-Methylpyrazino[2',3':4,5]thieno[3,2-d]pyrimidin-4(3H)-one (174).† In 7 ml of 95% ethanol was mixed 0.2 g (0.91 mmol) of 173 and 2 ml of concentrated ammonium hydroxide and the solution was refluxed for 4 hr. The yellow solution was cooled to room temperature and filtered to yield a white precipitate which was recrystallized from absolute ethanol to give 0.18 g (90%) of white crystals, mp 243-244°.

Anal. Calcd. for $C_9H_6N_4OS \cdot \frac{1}{2}H_2O$: C, 47.57; H, 3.08. Found: C, 47.81; H, 3.22.

24. 7-Aminothieno[2,3-b]pyrazine-6-carboxamide (175).†§ A mixture of 1.5 g (10.8 mmol) of 161, 0.98 g (10.8 mmol) of mercaptoacetamide and 1.48 g (13.9 mmol) of sodium carbonate in 25 ml of absolute ethanol was refluxed for 2 hr, cooled, and then filtered to give a greenish-yellow precipitate. The precipitate was mixed with water, filtered and then recrystallized from methanol to yield 2.0 g (96%)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

of a yellow product, mp 284-286°; mass spectrum m/e (relative abundance): 194 (100, M^+), 178 (13), 177 (76), 150 (13), 149 (38), 123 (16), 122 (20), 106 (10), 72 (17.5), 52 (20), 45 (24), 44 (20), 28 (17), 18 (22).

Anal. Calcd. for $C_7H_6N_4OS$: C, 43.27; H, 3.11. Found: C, 43.41; H, 3.31.

25. 7-Amino-6-cyanothieno[2,3-b]pyrazine (176). To 10 ml of phosphorus oxychloride was added 1.0 g (5.15 mmol) of 175 and the mixture was refluxed for 5 hr. After cooling, the mixture was carefully poured over ice and then filtered to yield a brown cake which was dried and sublimed (180°, 1.5 mm) to yield 0.8 g (88%) of yellow crystals, mp 204-206°.

Anal. Calcd. for $C_7H_4N_4S$: C, 47.71; H, 2.28. Found: C, 47.99; H, 2,27.

26. Pyrazino[2',3':4,5]thieno[3,2-d]pyrimidin-2,4-(1H,3H)-dione (177).†

Method A: To a mixture of 15 ml of pyridine and 0.5 g (2.58 mmol) of 175 was carefully added 2 ml of ethyl chloroformate and the mixture was refluxed for 48 hr. After cooling, the solution was concentrated and then water was added to produce a yellow precipitate (0.4 g, 70%) which was filtered and purified by repeated dissolution in aqueous base and precipitation by dilute acid, mp >340°.

Anal. Calcd. for $C_8H_4N_4O_2S \cdot H_2O$: C, 40.33; H, 2.52. Found: C, 40.46; H, 2.43.

Method B: A finely ground mixture of 0.4 g of 175 or

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

172 (2.1 mmol) and 0.8 g of urea were heated at 180° for 20 min. At this temperature the mixture melted and re-solidified. The solid mass was extracted with warm 5% sodium hydroxide, filtered and the cooled filtrate acidified with acetic acid to yield, upon filtering and air drying, a yellow product (60-70%) identical to that described in Method A.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

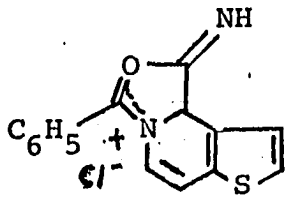
SELECTED SPECTRA

Infrared Spectra. A Perkin-Elmer Model 337 Spectrophotometer was used to record all the infrared spectra. All solids were measured as potassium bromide mulls and all liquids measured as liquid films. All spectra were calibrated using polystyrene film.

Nuclear Magnetic Resonance Spectra. The nuclear magnetic resonance spectra were obtained with a Varian Associates Model A-60 Nuclear Magnetic Resonance Spectrometer. The solvents used are indicated on the respective spectra. Tetramethylsilane was used as the internal standard for all samples. Where applicable, the offset (in Hertz) is indicated in parentheses in the upper left corner of the spectrum.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 1.



104

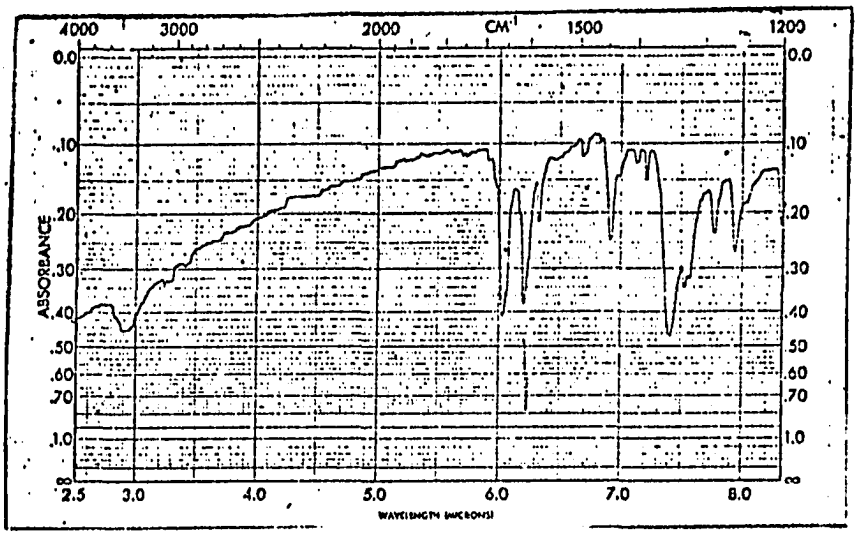
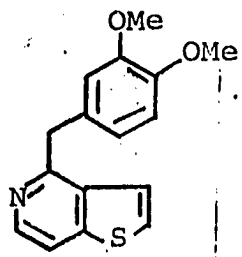


Figure 2.



109

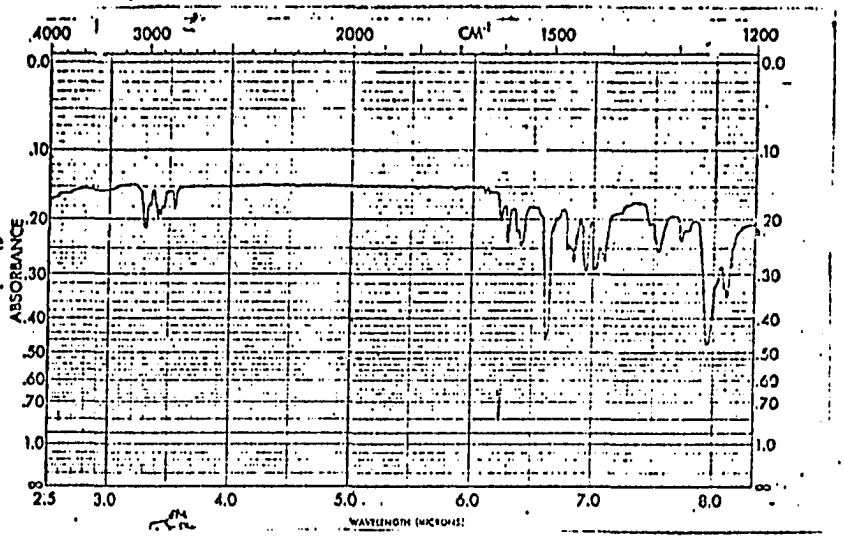
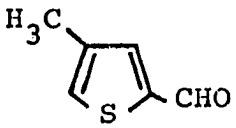
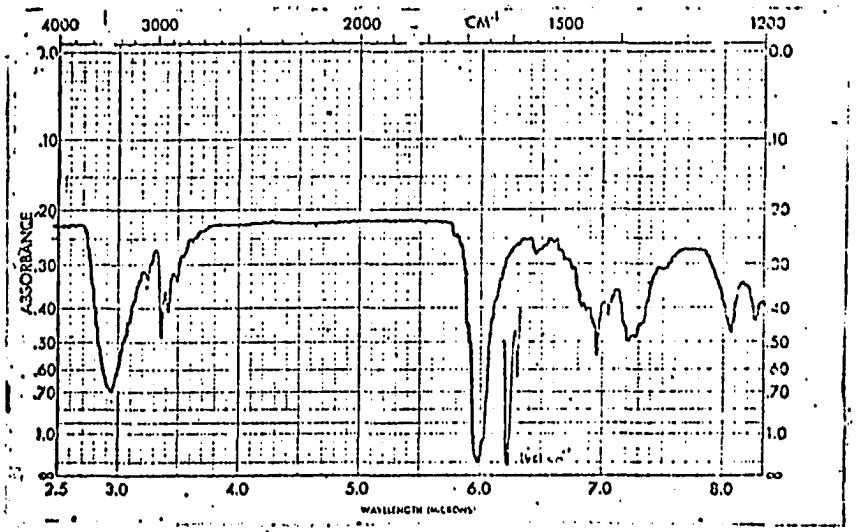


Figure 3.

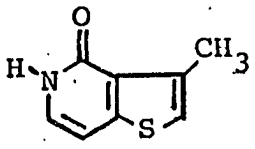


96



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 4.



99

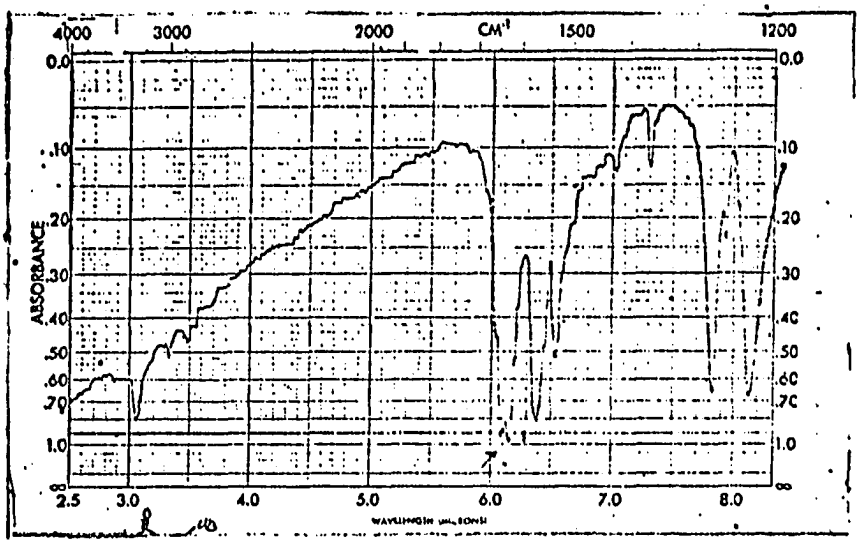
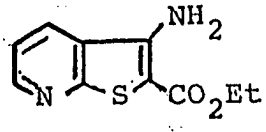


Figure 5.



116

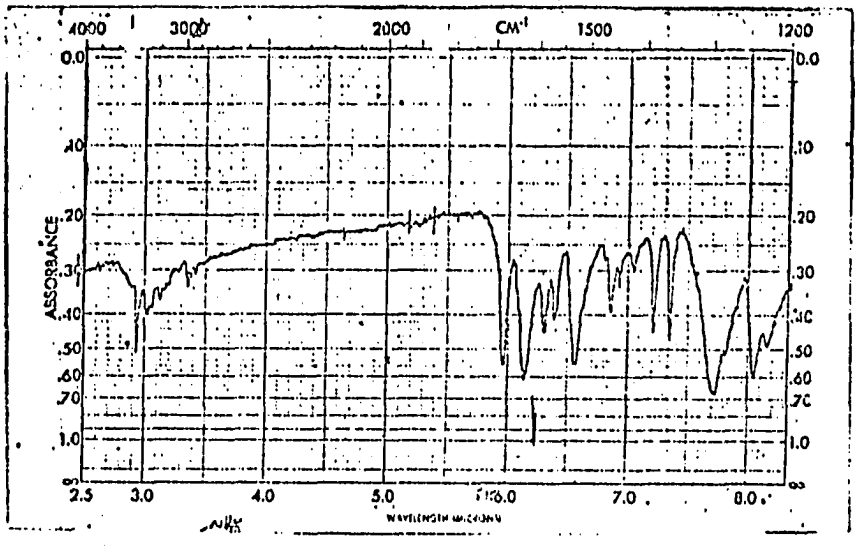
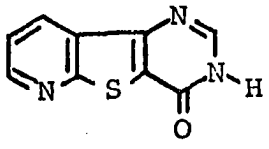
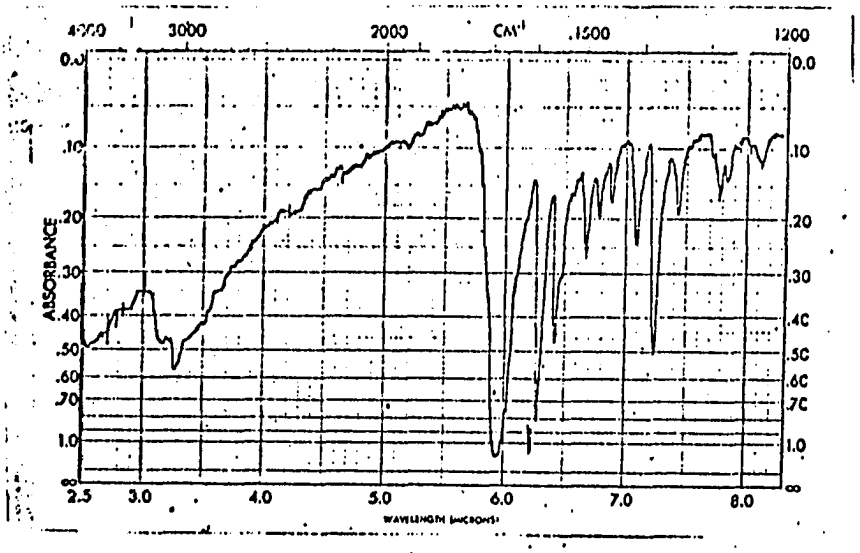


Figure 6.

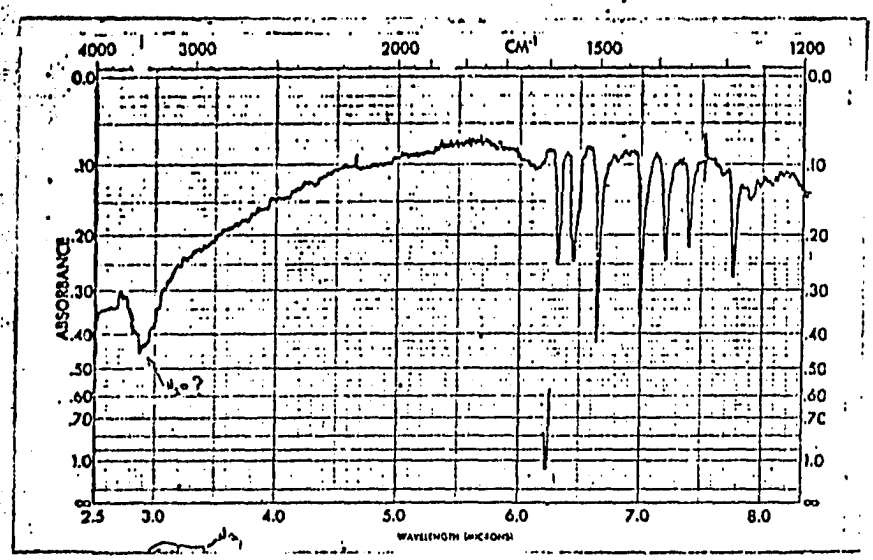


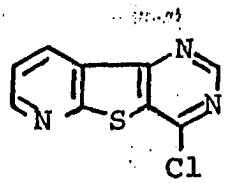
128



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

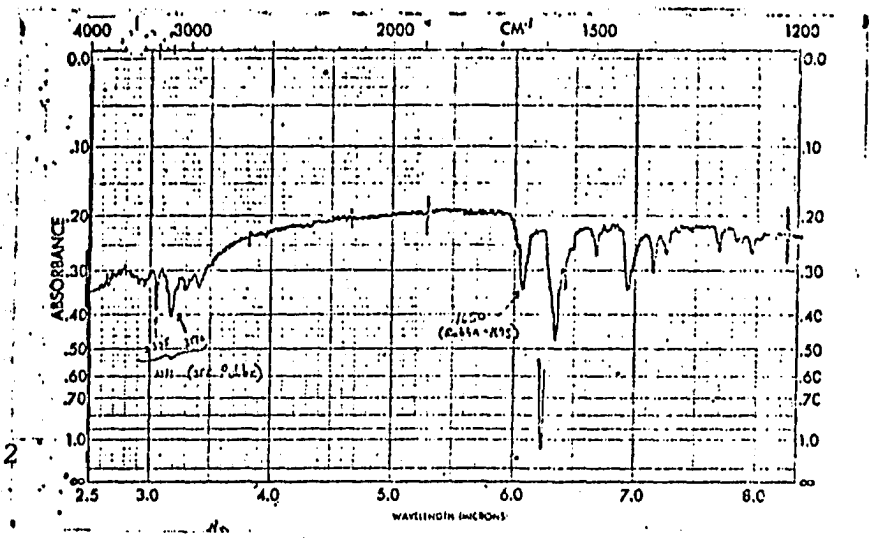
Figure 7.

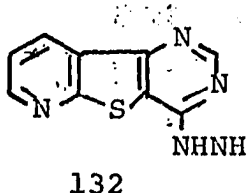




129

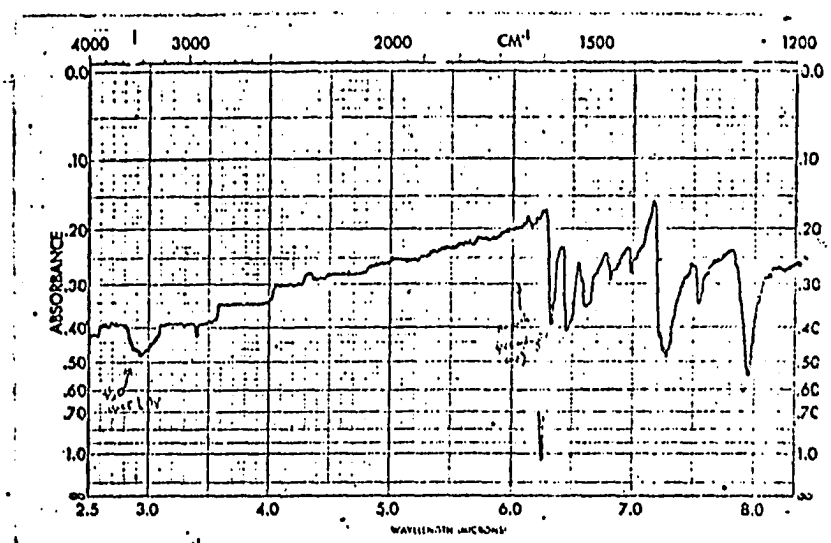
Figure 8.

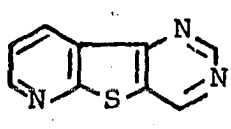




132

Figure 9.

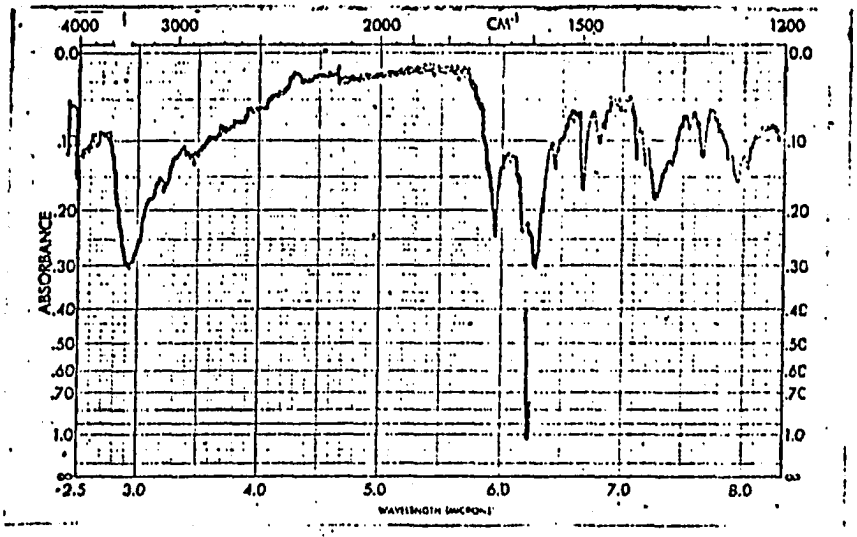


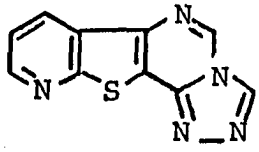


136

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

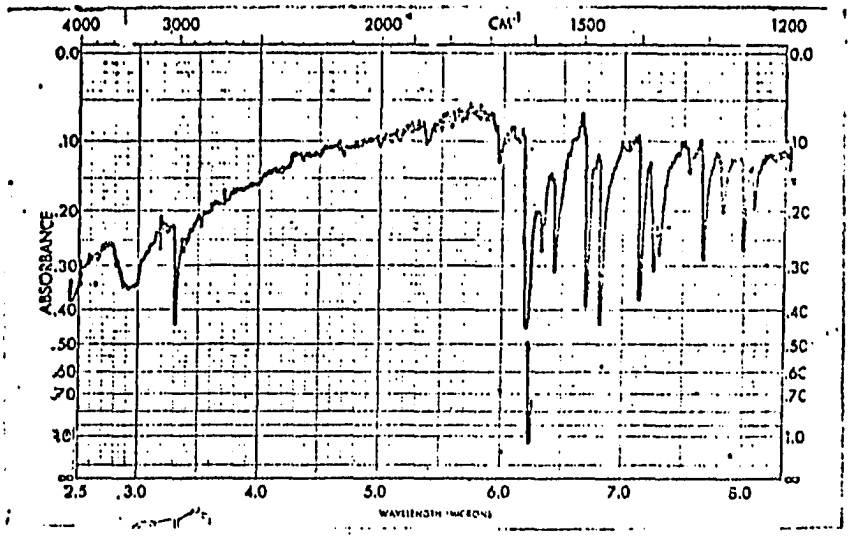
Figure 10.

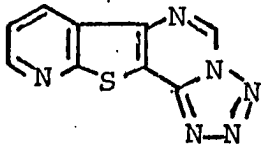




134

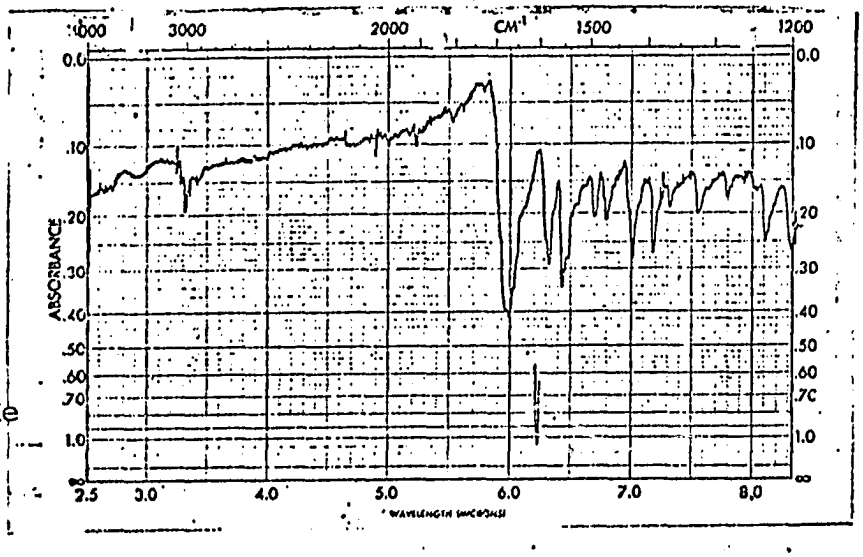
Figure 11.

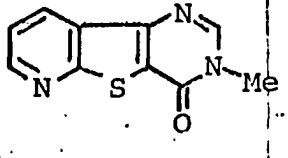




135

Figure 12.

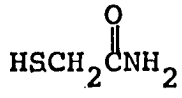




141

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 13.



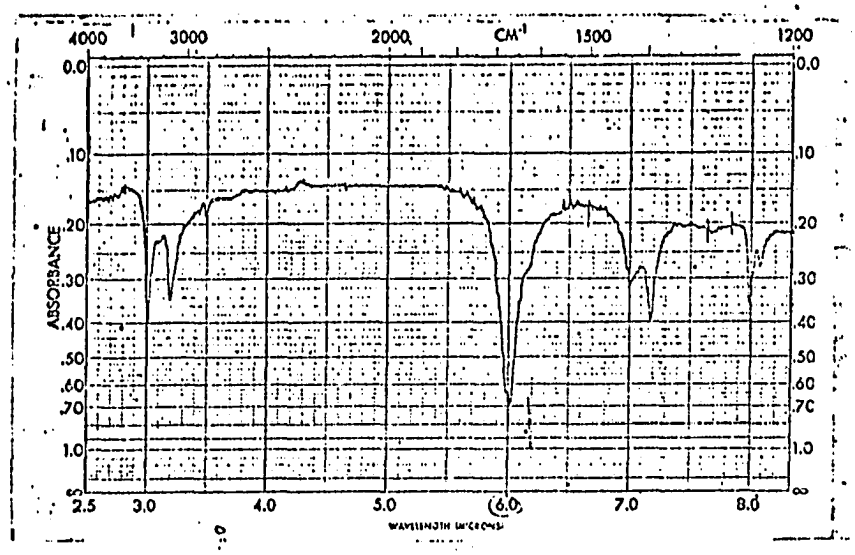
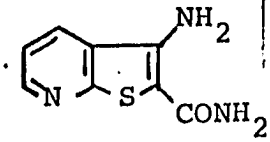


Figure 14.



155

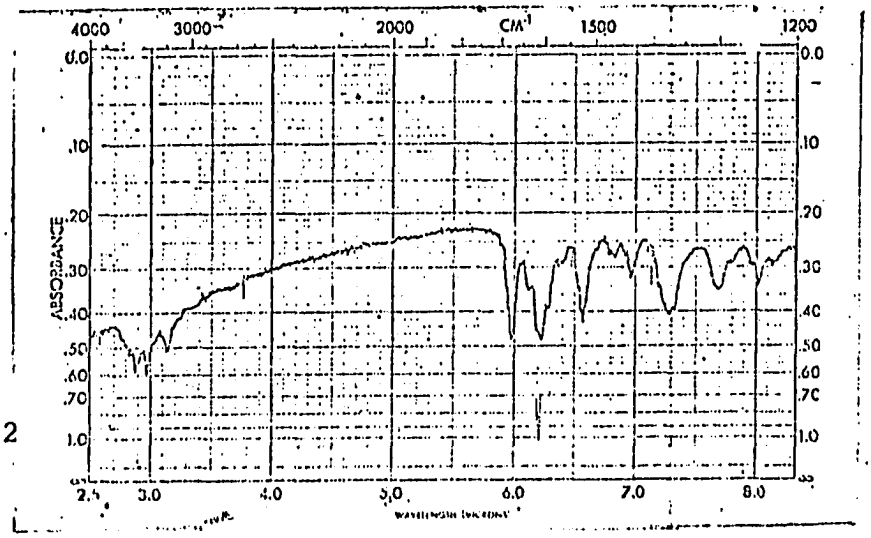
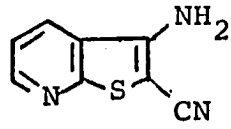
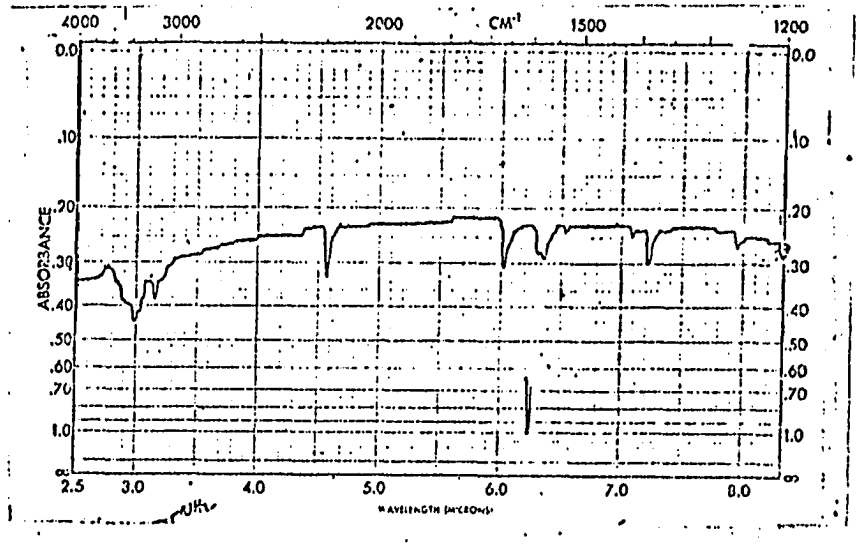


Figure 15.

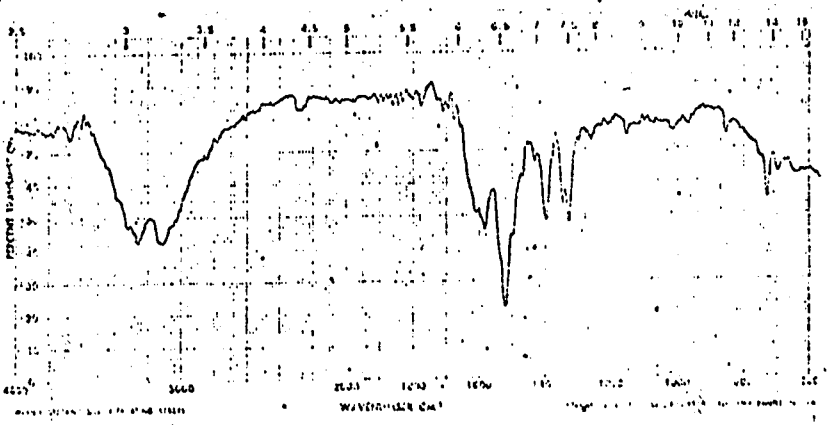


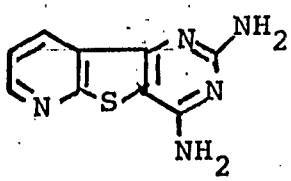
157



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

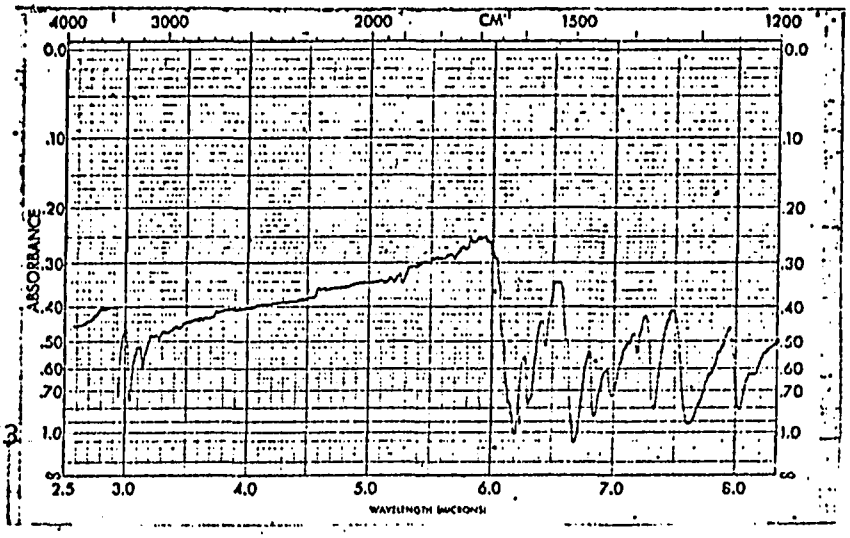
Figure 16.

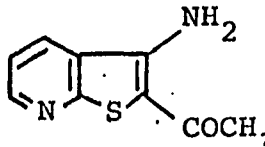




158

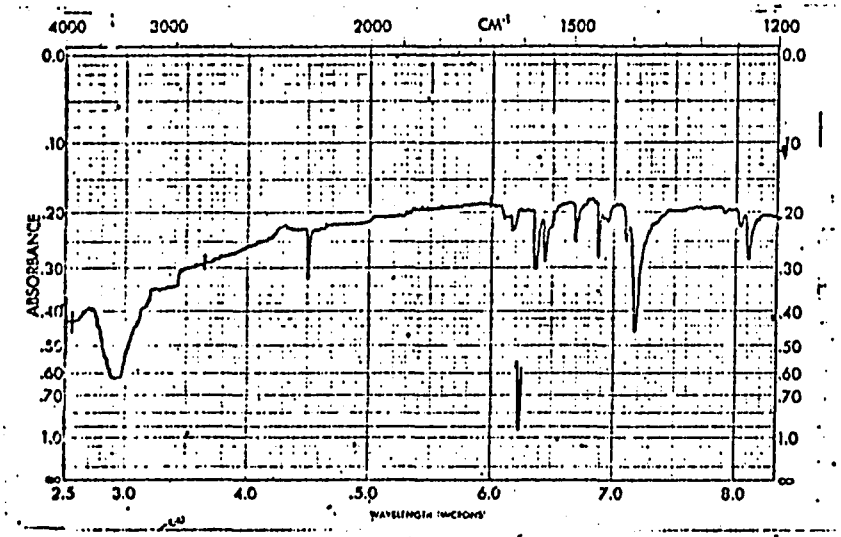
Figure 17.

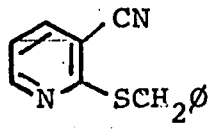




145

Figure 18.

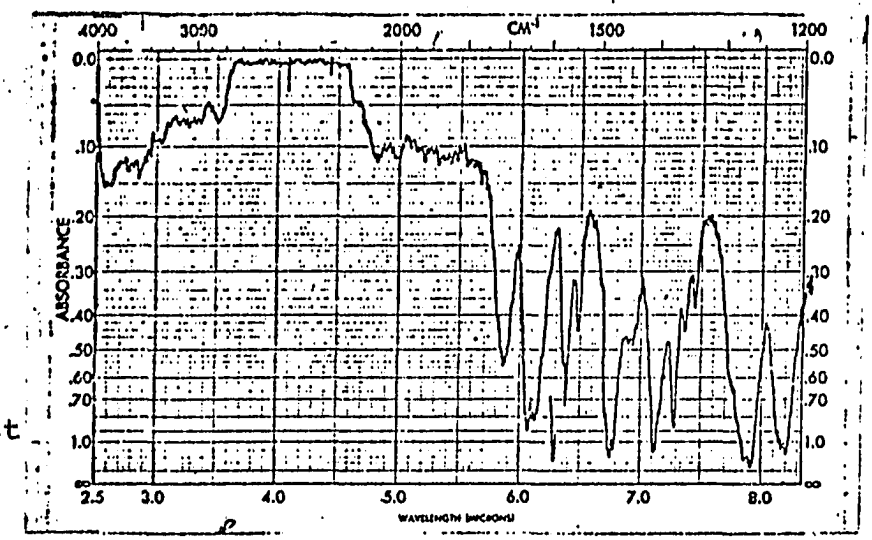


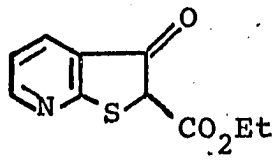


146

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

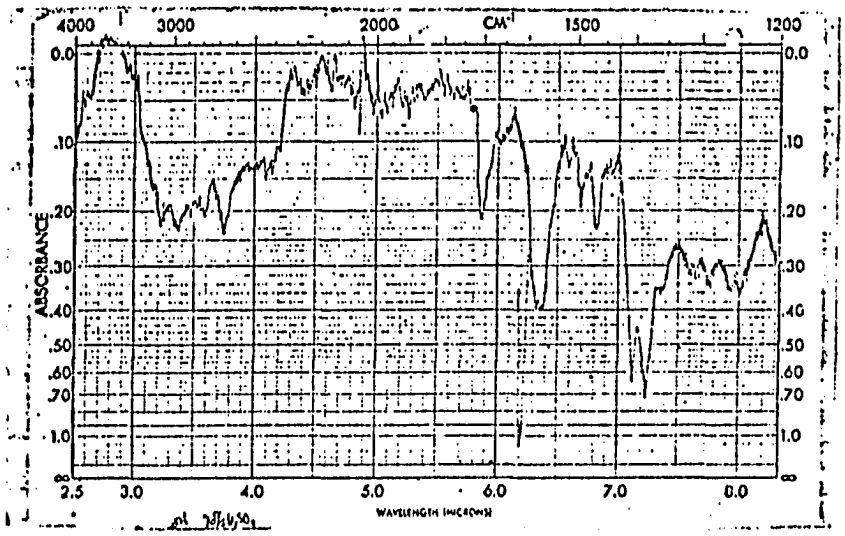
Figure 19.

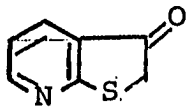




153

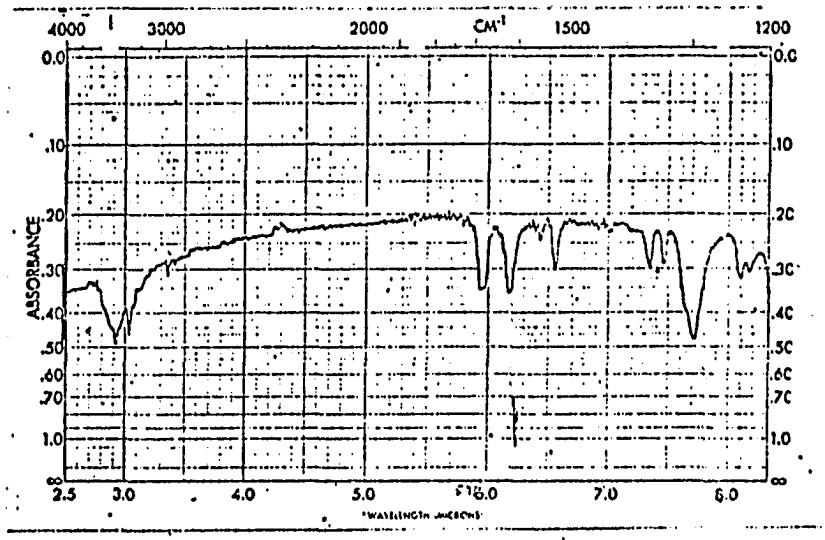
Figure 20.

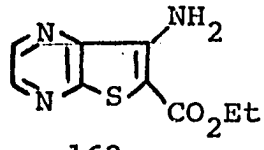




154

Figure 21.

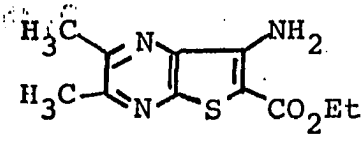




162

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 22.



163

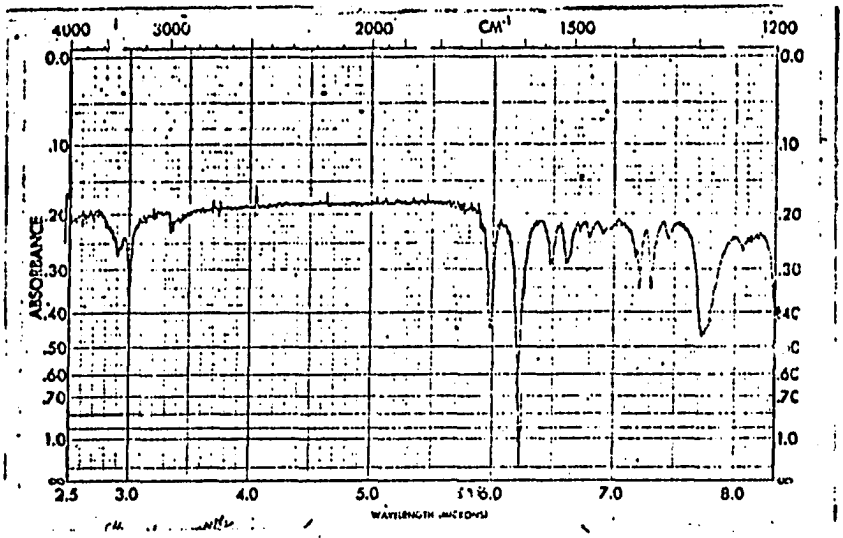
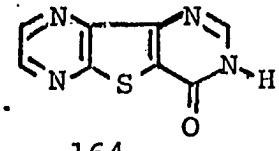


Figure 23.



164

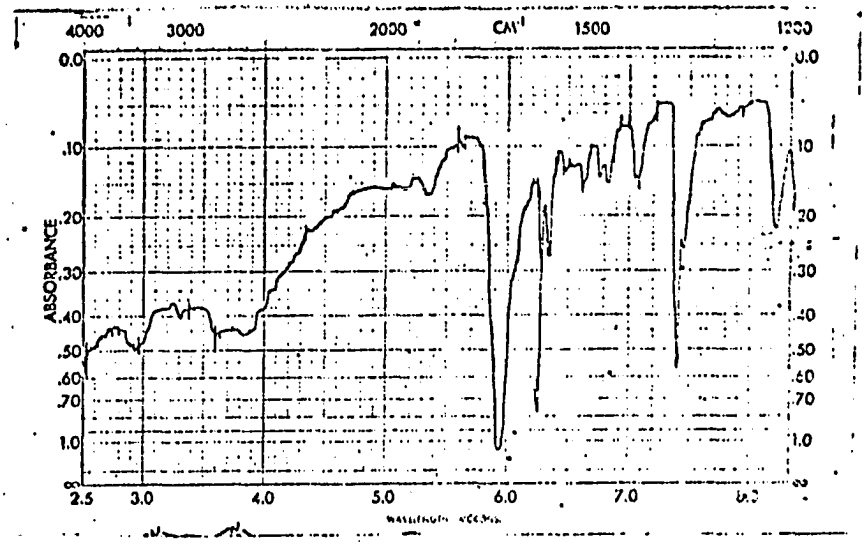
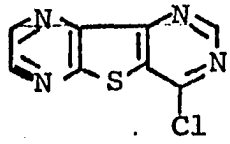
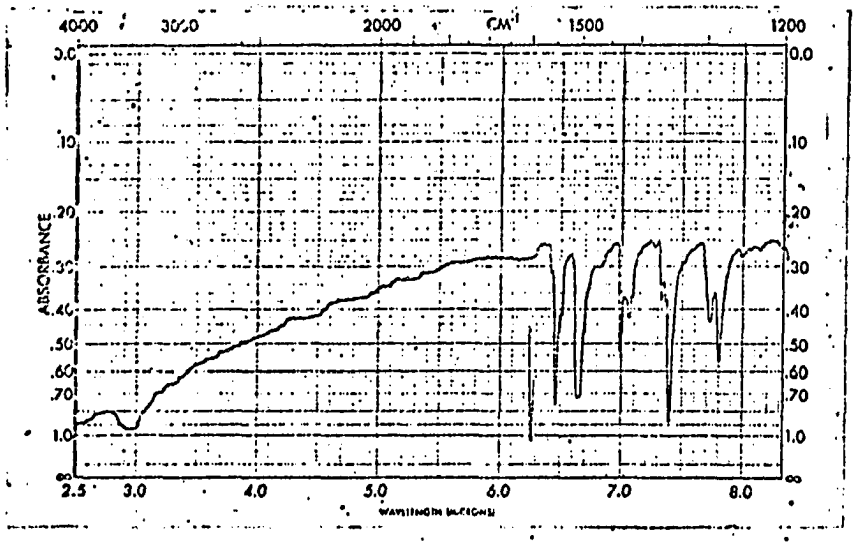


Figure 24.

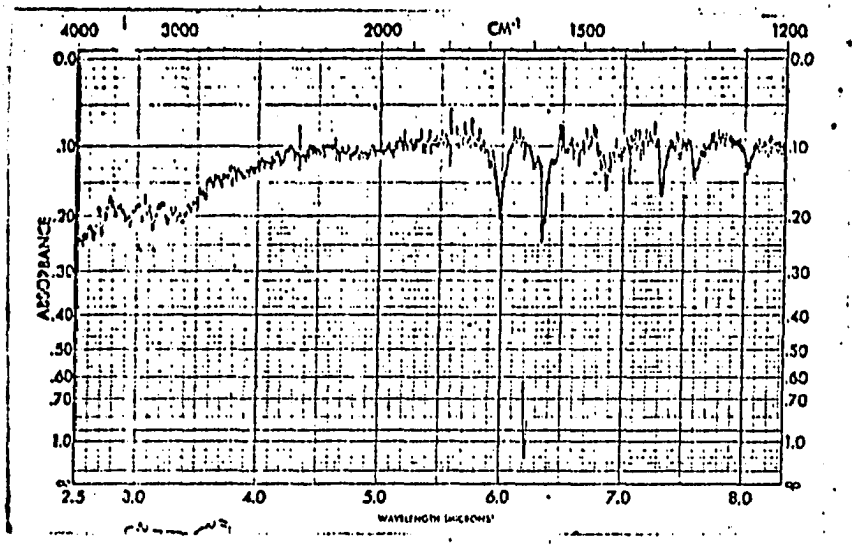


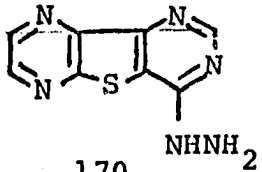
165



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

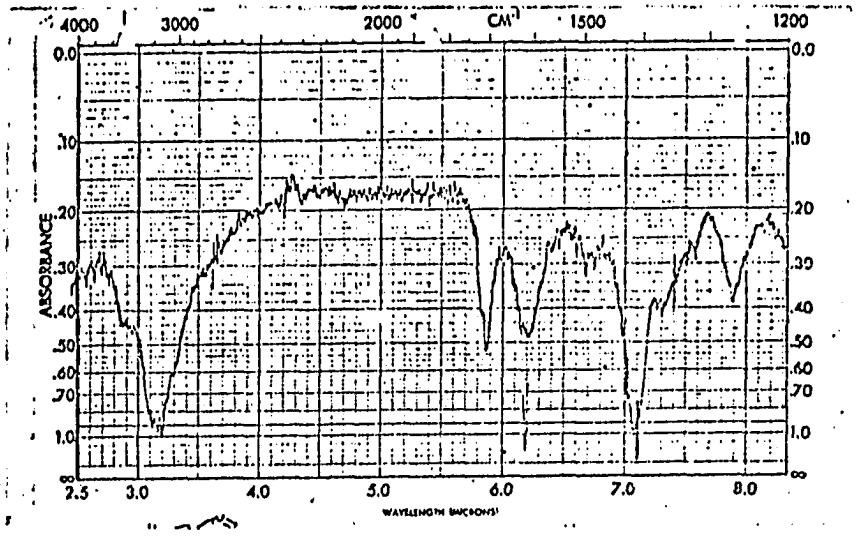
Figure 25.

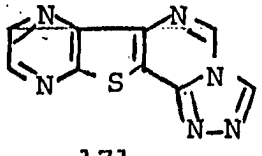




170

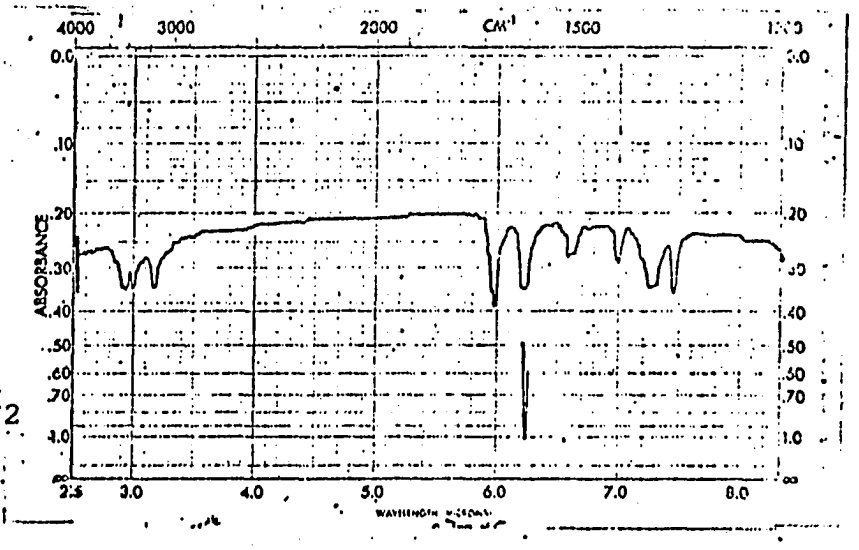
Figure 26.

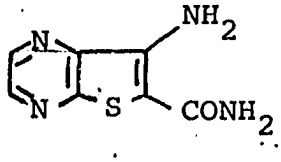




171

Figure 27.

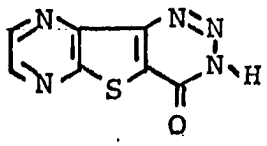




175

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 28.



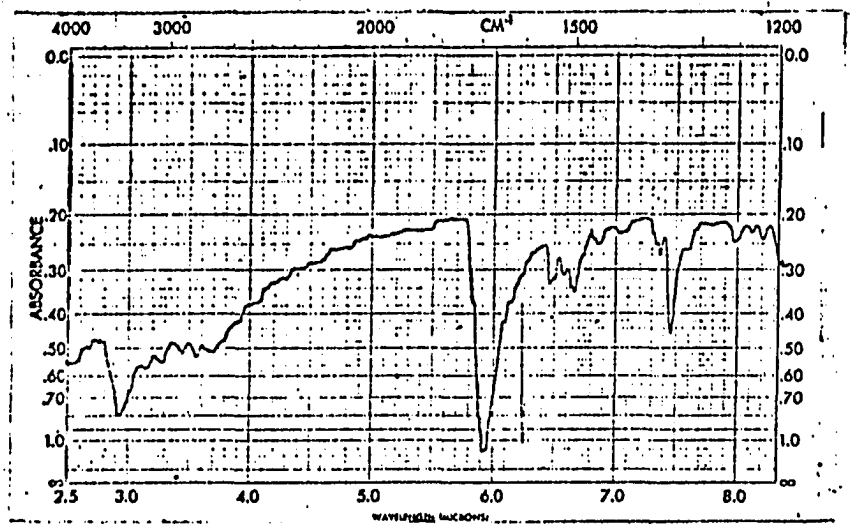
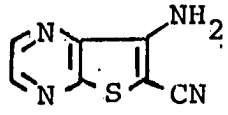


Figure 29.



176

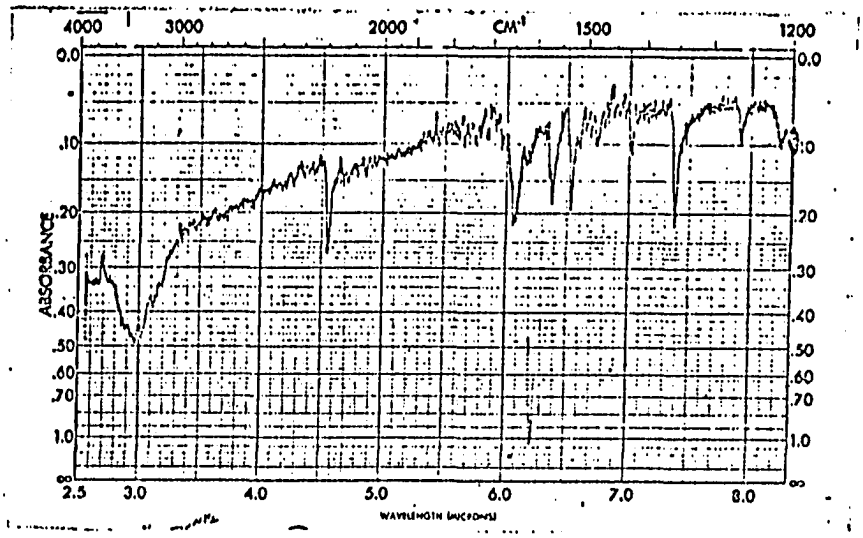
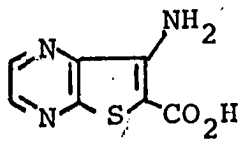
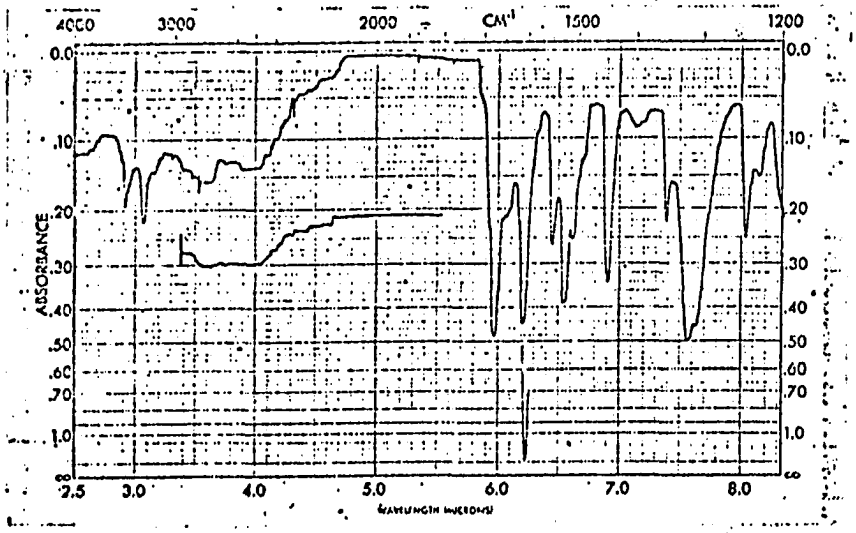


Figure 30.

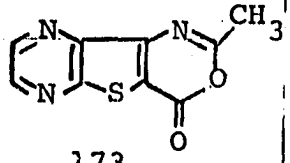


172



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 31.



173

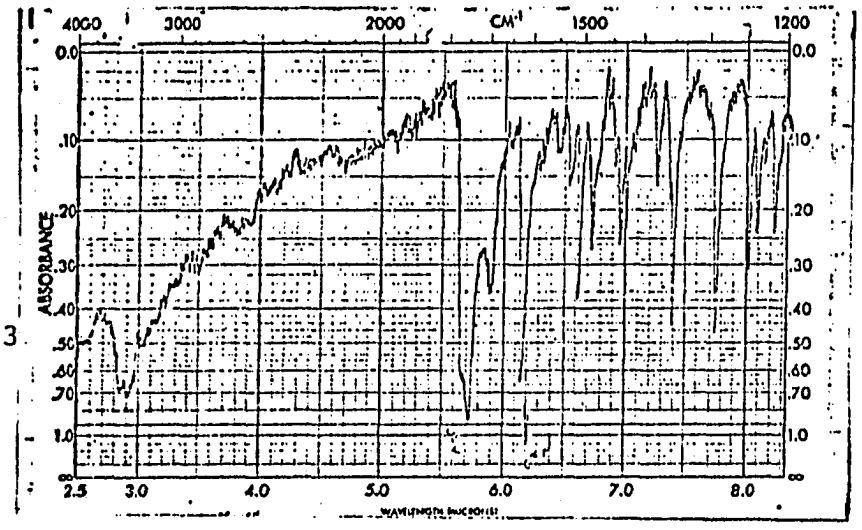
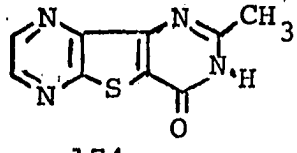


Figure 32.



174

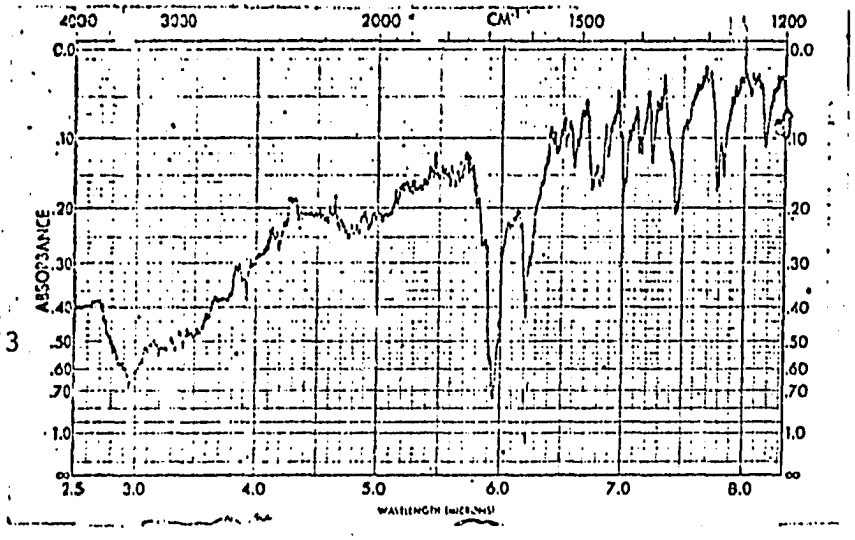
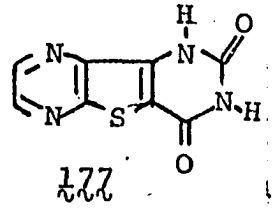
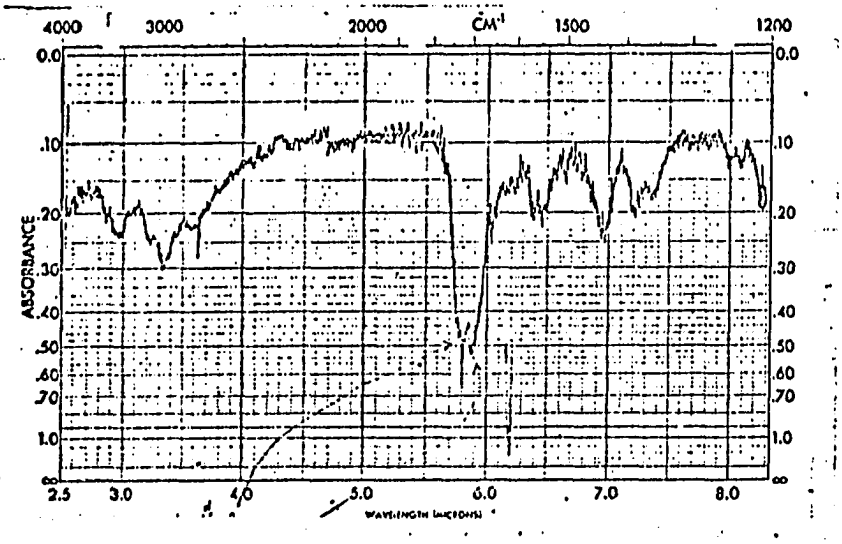


Figure 33.

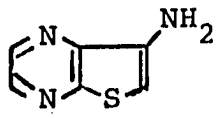


177



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 34.



178

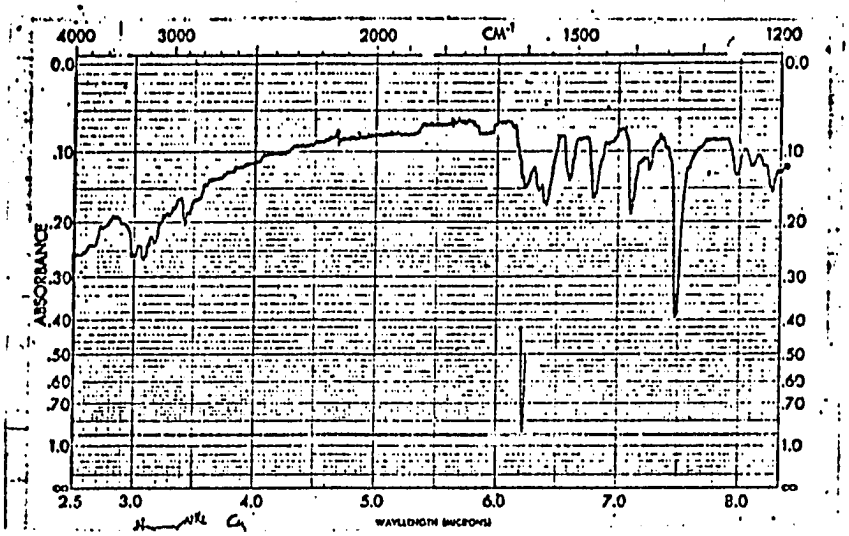
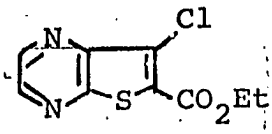


Figure 35.



182

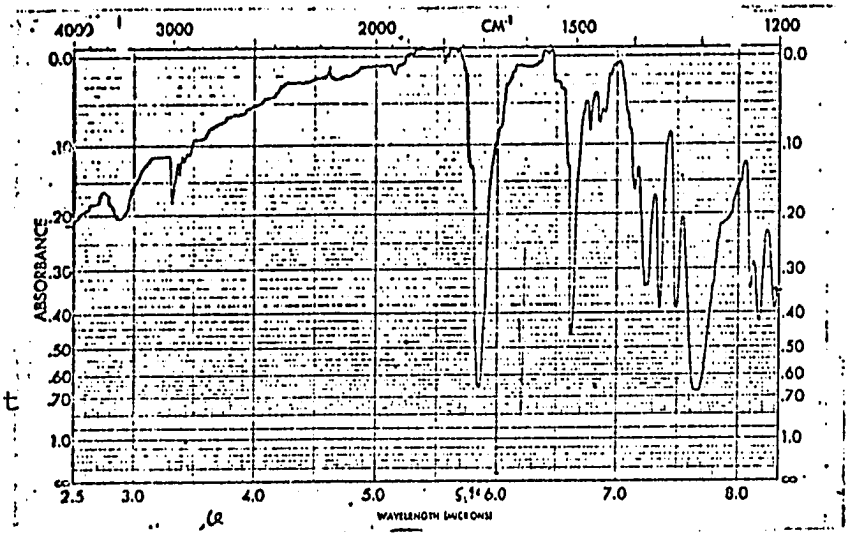
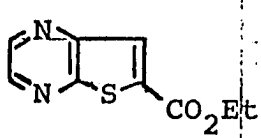
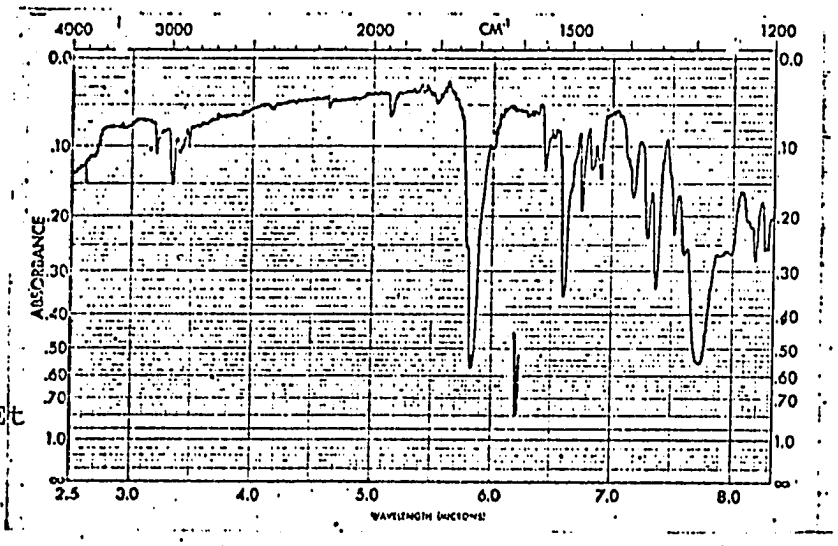


Figure 36.

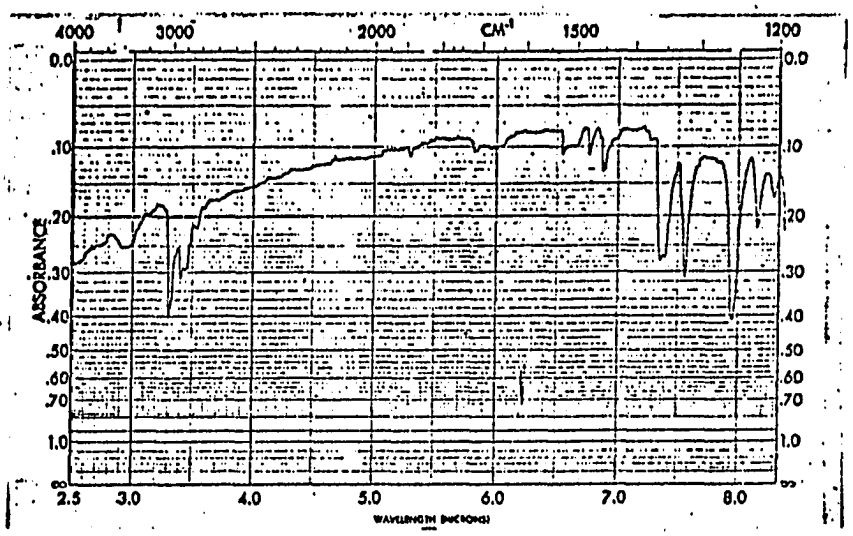


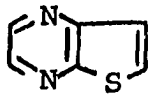
181



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 37.

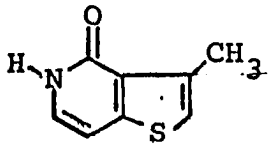




86

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 38.
($DMSO-d_6$)



99

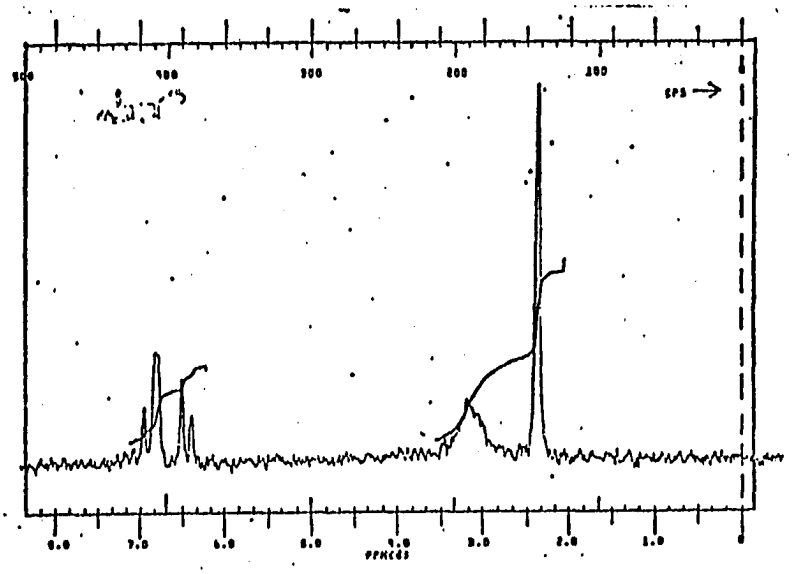
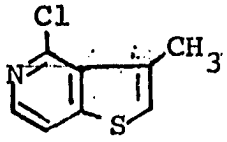


Figure 39.
($CDCl_3$)



100

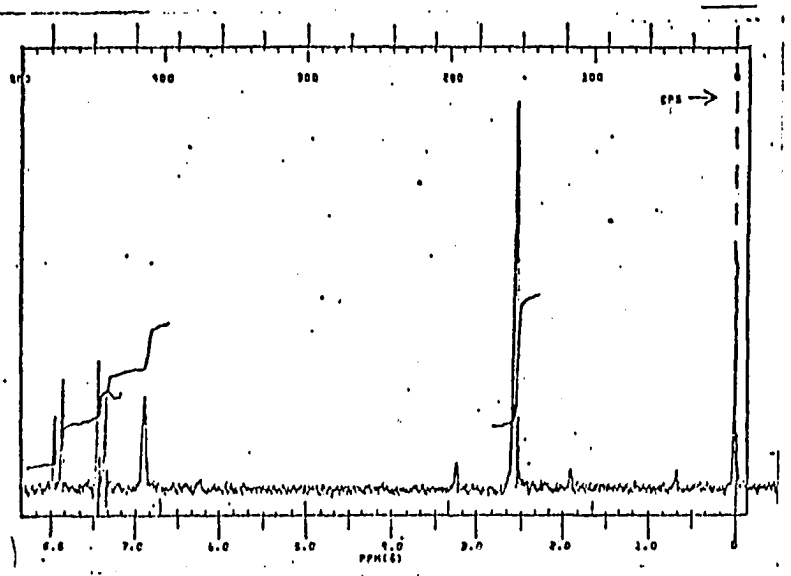
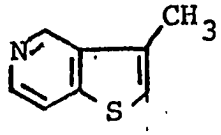
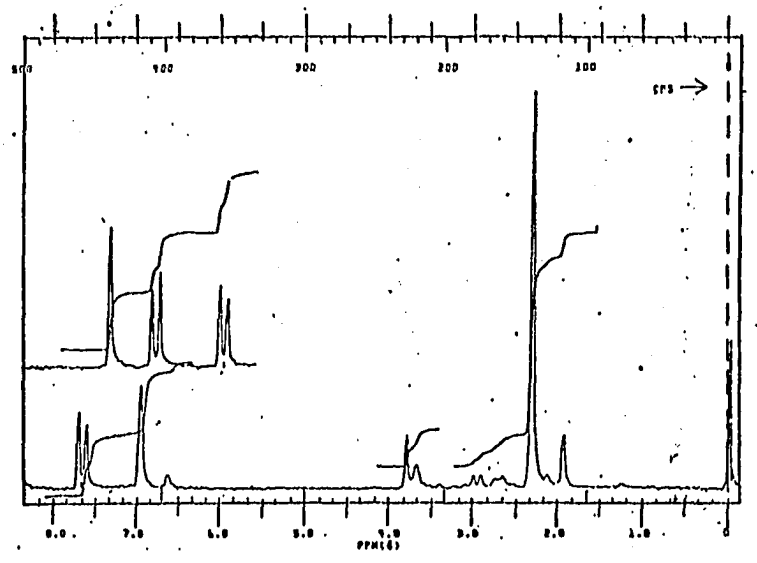


Figure 40.
($CDCl_3$)

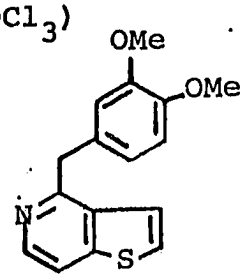


101



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 41.
($CDCl_3$)



112

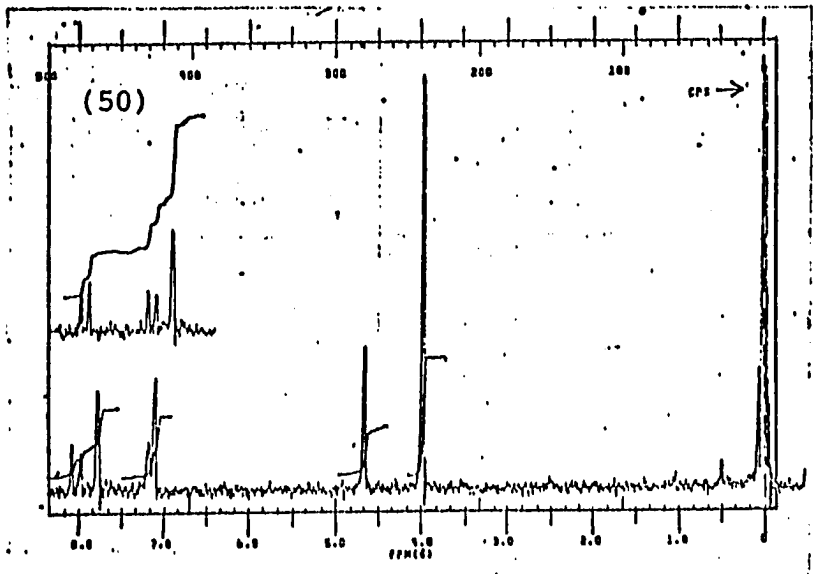
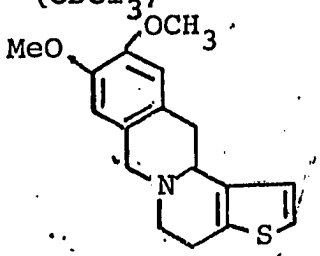


Figure 42.
($CDCl_3$)



113

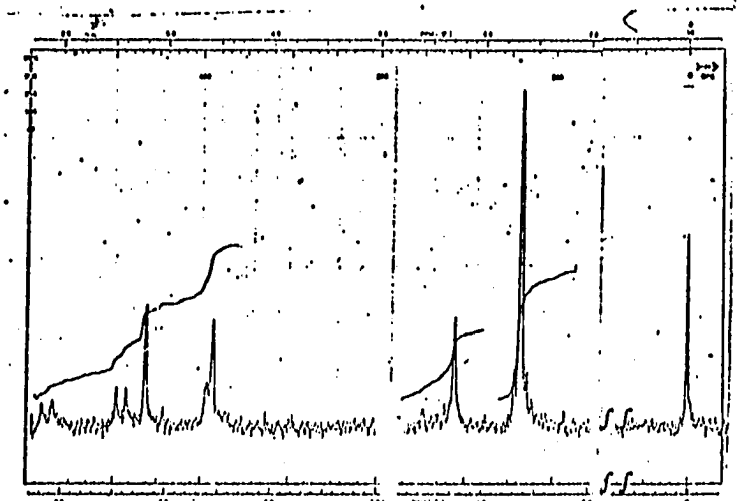
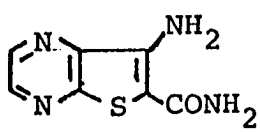
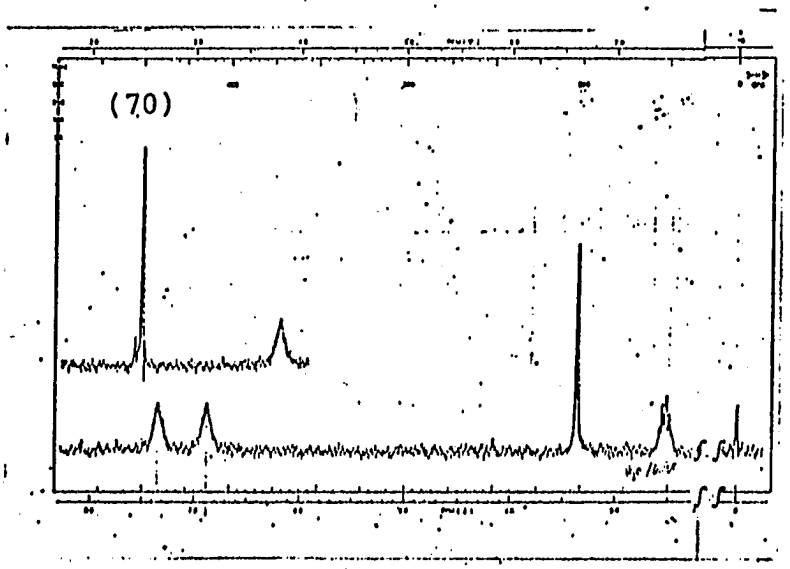


Figure 43.
($DMSO-d_6$)

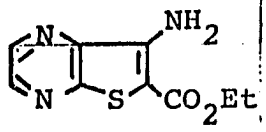


175



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 44.
($CDCl_3$)



162

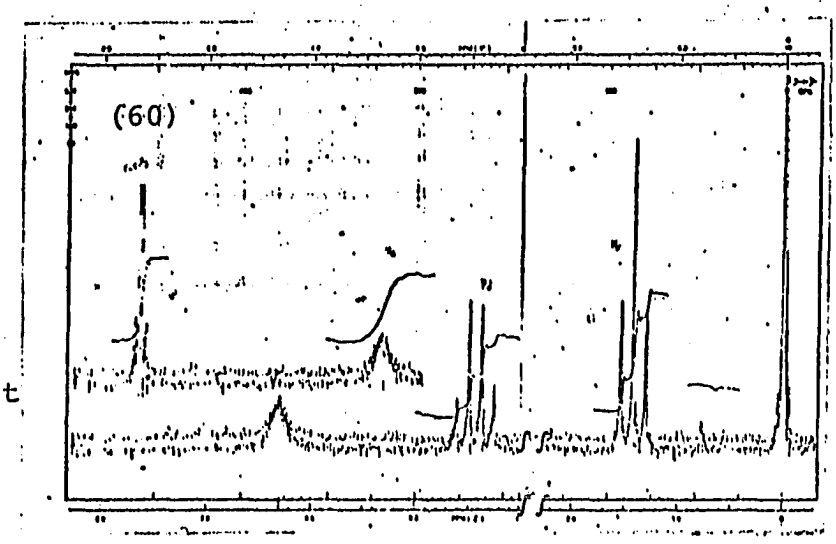
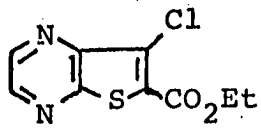


Figure 45.
($CDCl_3$)



182

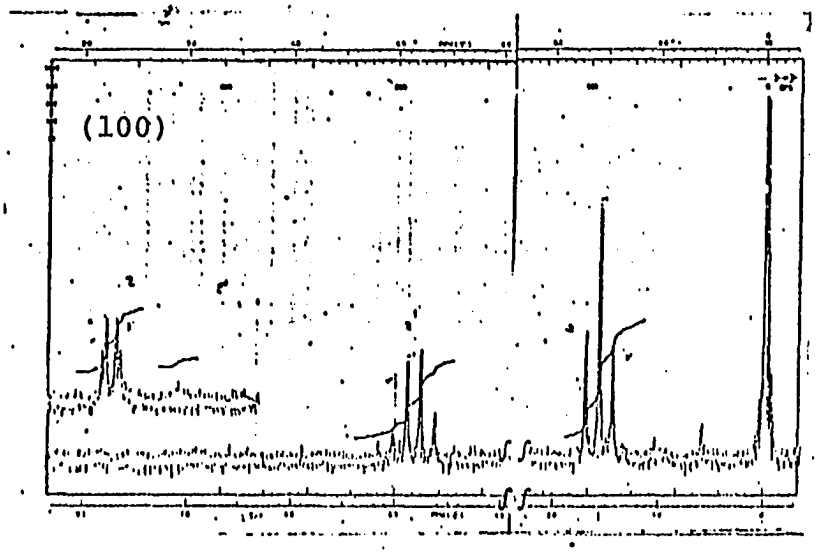
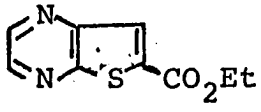
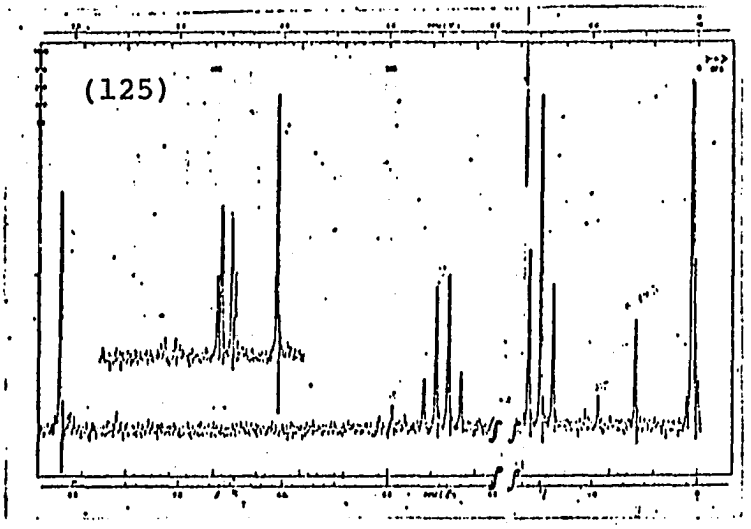


Figure 46.
($CDCl_3$)

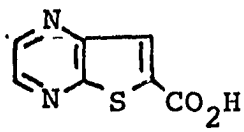


181



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 47.
(DMSO-d_6)



183

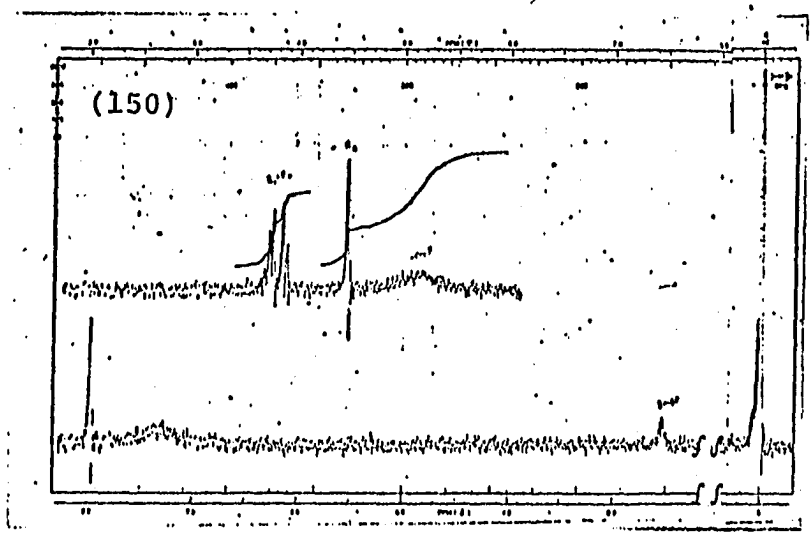
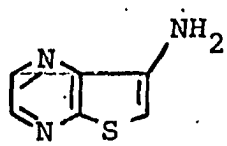


Figure 48.
($CDCl_3$)



178

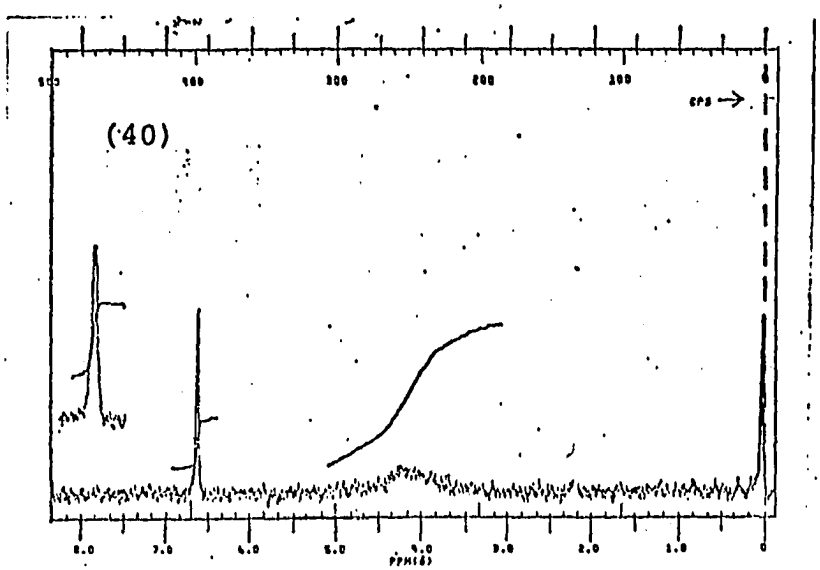
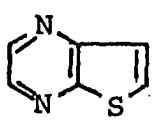
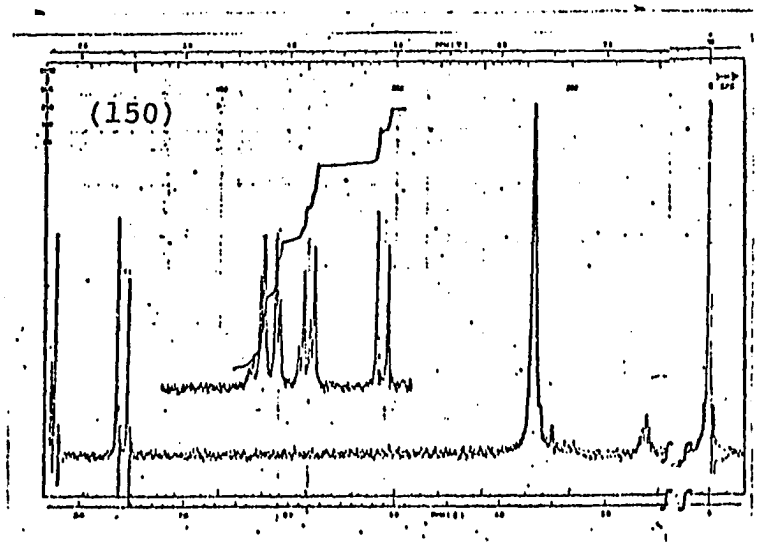


Figure 49.
(DMSO-d_6)

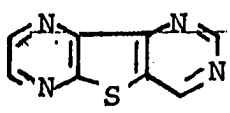


86



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 50.
(DMSO-d_6)



159

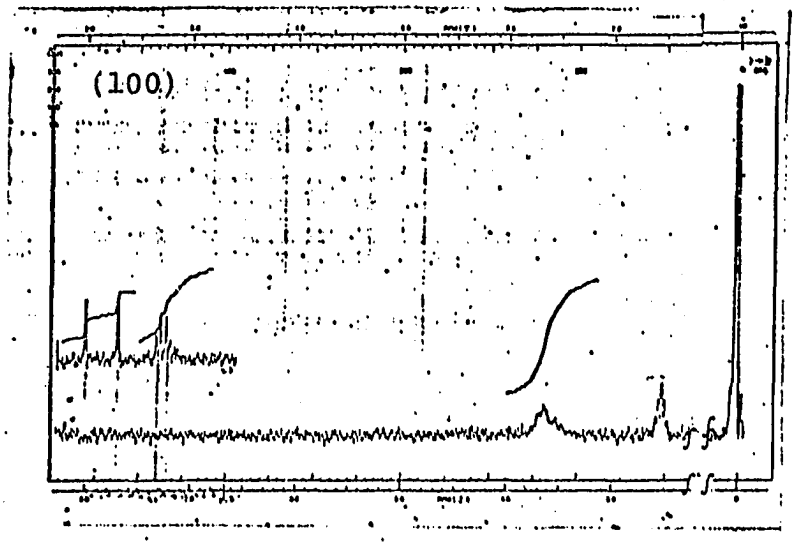
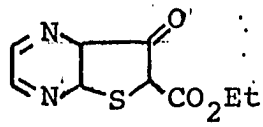
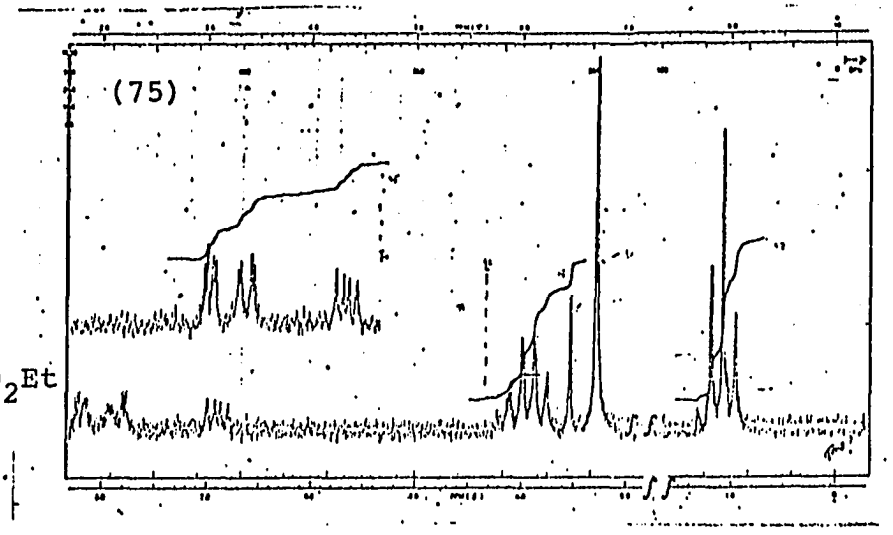


Figure 51.
(DMSO-d_6)



153



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

REFERENCES

1. A. Burger in *Medicinal Chemistry*, Ed., A. Burger, John Wiley and Sons, Inc., New York, 1970, p. 64.
2. W.T. Comer and A.W. Gomoll in *Medicinal Chemistry*, Ed., A. Burger, John Wiley and Sons, Inc., New York, 1970, p. 1019.
3. R.L. Noble, C.T. Beer and J.H. Cutts, *Biochem. Pharmacol.*, 1, 347 (1958).
4. F.W. Schueler, Ed., *Molecular Modification in Drug Design*, Advances in Chemistry Series, No. 45, American Chemical Society, Washington, D.C., 1964.
5. M. Tishler in *Molecular Modification in Drug Design*, Ed., F.W. Schueler, Advances in Chemistry Series, No. 45, American Chemical Society, Washington, D.C., 1964.
6. B.H. Takman and G. Camougis in *Medicinal Chemistry*, A. Burger, Ed., John Wiley and Sons, Inc., New York, 1970, p. 1607.
7. E.J. Ariens, *Molecular Pharmacology*, Academic Press, Inc., New York, 1964.
8. D.D. Woods, *Brit. J. Exper. Path.*, 21, 74 (1940).
9. E.F. Elslager and J. Davoll, *Lectures in Heterocyclic Chemistry*, Vol. II, S-97 (1974).
10. A. Albert, *Selective Toxicity*, John Wiley and Sons, Inc., New York, 1960.
11. I. Langmuir, *J. Amer. Chem. Soc.*, 41, 1543 (1919).
12. O. Hinsberg, *J. prakt. Chem.*, 93, 302 (1916).
13. H.G. Grimm, *Z. Electrochem.*, 31, 474 (1925); *idem.*, *Naturwissenshaften*, 17, 557 (1929).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

14. H. Erlenmeyer and E. Berger, *Biochem. Z.*, 252, 22 (1932); 255, 429 (1932); 262, 196 (1933).

15. H. Erlenmeyer and E. Berger, *Helv. Chim. Acta.*, 16, 733 (1933).

16. H.L. Freidman, *Influence of Isosteric Replacement Upon Biological Activity*, Symposium on Chemical-Biological Correlation, Nat'l Acad. Sci., -Nat'l Research Council, Publ. No. 206, Washington, D.C., 1951.

17. M.M. Rapport, A.A. Green and I.H. Page, *J. Biol. Chem.*, 174, 735 (1948).

18. M.M. Rapport, *J. Biol. Chem.*, 180, 961 (1949).

19. K.E. Hamlin and F.E. Fischer, *J. Amer. Chem. Soc.*, 73, 5007 (1951).

20. V. Erspamer and B. Asero, *Nature (London)*, 169, 800 (1952).

21. S. Garratini and L. Valzelli, *Serotonin*, Elsevier, Amsterdam, 1965.

22. V. Erspamer, Ed., *5-Hydroxytryptamine and Related Indolealkylamines*, Vol. 19 of *Handbook of Experimental Pharmacology*, Springer, Berlin, 1966.

23. Symposium: *Biological Role of Indolealkylaamine Derivatives*, New York Academy of Sciences, May 10-12, 1967.

24. W.W. Douglas in *The Pharmacological Basis of Therapeutics*, 3rd ed., L.S. Goodman and A. Gilman, Eds., The Macmillan Co., New York, 1965, Chapter 30.

25. K. Freter, H. Weissbach, B. Redfield, S. Udenfriend and B. Witkop, *J. Amer. Chem. Soc.*, 80, 983 (1958).

26. S. Udenfriend, C.R. Creveling, H. Posner, B.G. Redfield, J. Daly and B. Witkop, *Arch. Biochem. Biophys.*, 83, 501 (1959).

27. S. Udenfriend, H. Weissbach and D.F. Bogdanski, *Ann. N.Y. Acad. Sci.*, 66, 602 (1957).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

28. D.F. Bogdanski and S. Udenfriend, *J. Pharm. Exptl. Therap.*, 122, 182 (1958).

29. G. Guroff and S. Udenfriend, *J. Biol. Chem.*, 237, 803 (1962

30. B.B. Brodie and M.A. Beaven, *Med. Exptl.*, 8, 320 (1963).

31. D.J. Triggle in *Medicinal Chemistry*, A. Burger, Ed., John Wiley and Sons, Inc., New York, 1970, p. 1235.

32. J.R. Vane, *Brit. J. Pharmacol.*, 14, 87 (1959).

33. J.C. Winter, P.K. Gessner and D.D. Godse, *J. Med. Chem.*, 10, 856 (1967).

34. R.B. Barlow and I. Khan, *Brit. J. Pharmacol.*, 14, 99 (1959).

35. R.B. Barlow, *Brit. J. Pharmacol.*, 16, 153 (1961).

36. R.E. Handschmacher and J.R. Vane, *Brit. J. Pharmacol.*, 29, 105 (1967).

37. R.B. Angier, J.H. Booth, B.L. Hutchins, J.H. Mowat, J. Semb, E.R.L. Stokstad, ... Subbarow, C.W. Waller, D.B. Cosulich, M.J. Fahrenbach, M.E. Hultquist, E. Kuh, E.H. Northey, D.R. Seeger, J.P. Sickels, and J.M. Smith, Jr., *Science*, 103, 667 (1946).

38. F.M. Huennekens, *Folate and B_{12} Coenzymes*, in *Biological Oxidations*, T.P. Singer, Ed., Interscience Publishers, New York, 1968, pp. 439-482.

39. R.L. Blakley, *The Biochemistry of Folic Acid and Related Pteridines*, in, *Frontiers of Biology*, Vol. 13, A. Neuberger and E.L. Tatum, Eds., North-Holland Publishing Co., Amsterdam, Netherlands, 1969.

40. J.R. Bertino, Ed., *Folate Antagonists as Chemotherapeutic Agents*, in, *Ann. N.Y. Acad. Sci.*, 186, 5 (1971).

41. G.H. Hitchings and J.J. Burchall, *Inhibition of Folate*

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Biosynthesis and Function as a Basis for Chemotherapy, in, *Advances in Enzymology*, Vol. 27, F.F. Nord, Ed., Interscience Publishers, New York, 1965, pp. 417-468.

42. D.R. Seeger, D.B. Cosulich, J.M. Smith, Jr., and M.E. Hultquist, *J. Amer. Chem. Soc.*, 71, 1753 (1949).

43. H.C. Carrington, A.F. Crowther, D.G. Davey, A.A. Lewis, and F.L. Rose, *Nature*, 168, 1080 (1951).

44. *World Health Organization Technical Report Service*, No. 226 (1961).

45. W. Peters, *Chemotherapy and Drug Resistance in Malaria*, Academic Press, New York, 1970.

46. H.T. Openshaw in *The Alkaloids*, R.H.T. Manske and H.L. Holmes, Eds., Vol. III, Academic Press, New York, 1953, pp. 101-118.

47. E. Kremers, *Proc. Celebration 300th Anniversary of the First Recognized Use of Cinchona*, St. Louis, 139 (1931).

48. R.M. Pinder in *Medicinal Chemistry*, A Burger, Ed., John Wiley and Sons, Inc., New York, 1970, p. 492.

49. C. Temple, Jr., C.L. Kussner and J.A. Montgomery, *J. Org. Chem.*, 39, 2866 (1974).

50. J. A. Montgomery in *Medicinal Chemistry*, A. Burger, Ed., John Wiley and Sons, New York, 1970, 680.

51. J. Davoll and A.M. Johnson, *J. Chem. Soc., C*, 997 (1970).

52. D.D. Bird, J.W. Vaitkus and J. Clarke, *Mol. Pharmacol.*, 6, 573 (1970).

53. J. Davoll, A.M. Johnson, H.J. Davies, O.D. Bird, J. Clarke and E.F. Elslager, *J. Med. Chem.*, 15, 812 (1972).

54. E.F. Elslager, J. Johnson, L.M. Werbel and J. Davoll, Abstracts of Papers, 163rd National Meeting of the American Chemical Society, Boston, Massachusetts, April 9-14, 1972, MEDI 36.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

55. E.F. Elslager, P. Jacob and L.M. Werbel, *J. Heterocyclic Chem.*, 9, 775 (1972).

56. A. Rosowsky, M. Chaykovsky, K.K.N. Chen, M. Lin and E.J. Modest, *J. Med. Chem.*, 16, 185 (1973); M. Chaykovsky, M. Lin, A. Rosowsky, and E.J. Modest, *ibid.*, 16, 188 (1973); A. Rosowsky, K.K.N. Chen, and M. Lin, *ibid.*, 16, 191 (1973).

57. B. Roth, *J. Med. Chem.*, 12, 227 (1969).

58. A. Rosowsky and E.J. Modest, *N.Y. Acad. Sci.*, 186, 280 (1971).

59. A. Rosowsky, K.K.N. Chen, M.E. Nadel, N. Papathanasopoulos and E.J. Modest, *J. Heterocycl. Chem.*, 9, 275 (1972); A. Rosowsky, K.K.N. Chen, N. Papathanasopoulos and E.J. Modest, *ibid.*, 9 263 (1972); A. Rosowsky, K.K.N. Chen, M.E. Nadel, R. St. Amand and S.A. Yeager, *ibid.*, 8, 789 (1971); and A. Rosowsky, J.L. Marini, M.E. Nadel and E.J. Modest, *J. Med. Chem.*, 13, 882 (1970).

60. M.G. Nair and C.M. Baugh, *J. Med. Chem.*, 17, 223 (1974).

61. R.F. Struck, Y.F. Shealy and J.A. Montgomery, *J. Med. Chem.*, 14, 693 (1971).

62. J.I. DeGraw, R.L. Kisliuk, Y. Gaumont, C.M. Baugh and M.G. Nair, *J. Med. Chem.*, 17, 552 (1974).

63. E.C. Roberts and Y.F. Shealy, *J. Med. Chem.*, 16, 697 (1973).

64. S.W. Schneller, *Int. J. Sulfur Chem., B*, 7, 309 (1972).

65. L.H. Klemm and D. Reed, *J. Org. Chem.*, 25, 1816 (1960).

66. L.H. Klemm, D.R. McCoy, J. Shabtai and W.K.T. King, *J. HeterocyclChem.*, 5, 883 (1968); *idem.*, 6, 813 (1969).

67. L.H. Klemm and D.R. McCoy, *J. Heterocycl. Chem.*, 6, 73 (1969).

68. L.H. Klemm, C.E. Klopfenstein, R. Zell, D.R. McCoy and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

R.A. Klemm, *J. Org. Chem.*, 34, 347 (1969).

69. W.S. Emerson, F.W. Holly and L.H. Klemm, *J. Amer. Chem. Soc.*, 63, 2569 (1941).

70. V.G. Zhiryakov and P.I. Abramenko, *Zhur. Vsesoyuz. Khim. Obshchestva im. D.I. Mendeleeva*, 5, 707 (1960); *Chem. Abstr.*, 55, 11416c (1961).

71. V.G. Zhiryakov and P.I. Abramenko, *Khim. Geterotsikl. Soedin. Akad. Nauk. Latv. SSR*, 334 (1965); *Chem. Abstr.*, 63, 13231g (1965).

72. P.I. Abramenko and V.G. Zhiryakov, *USSR Patent* 165,731 (1965); *Chem. Abstr.*, 62, P6488g (1965).

73. P.I. Abramenko, *Khim. Geterostikl. Soedin.*, 368 (1967); *Chem. Abstr.*, 67, 116827n (1967).

74. W.J. Raich and C.S. Hamilton, *J. Amer. Chem. Soc.*, 79, 3800 (1957).

75. J.T. Sheehan and G.J. Leitner, *J. Amer. Chem. Soc.*, 74, 5501 (1952).

76. W. Herz and L. Tsai, *J. Amer. Chem. Soc.*, 75, 5122 (1953).

77. W. Herz, *J. Amer. Chem. Soc.*, 73, 351 (1951).

78. W. Herz and L. Tsai, *J. Amer. Chem. Soc.*, 77, 3529 (1955).

79. M. Descamp and F. Binon, *Bull. Soc. Chim. Belges*, 71, 579 (1962).

80. M.L. Dressler and M.M. Joullie, *J. Heterocycl. Chem.*, 7, 1257 (1970).

81. S. Gronowitz and E. Sandberg, *Arkv. Kemi.*, 32, 217 (1970).

82. F. Eloy and A. Deryckere, *Bull. Soc. Chim. Belges*, 79 301 (1970).

83. C. Hansch, W. Carpenter and T. Todd, *J. Org. Chem.*, 23, 1924 (1958); *ibid.*, 22, 936 (1957).

84. S.V. Krivum, V.I. Dulenko, L.V. Dulenko and G.N.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Dorofunko, *Dolk. Akad. Nauk. SSSR*, 166, 359 (1966); *Chem. Abstr.*, 64, 11154b (1966); and L.V. Dulenko, G.N. Dorofenko, S.N. Baranov, I.G. Katts and V.I. Dulenko, *Khim. Geterotsikl. Soedin.*, 7, 320 (1971); *Chem. Abstr.*, 76, 14378k (1972).

85. K. Aparajithan, A.C. Thompson and J. Same, *J. Heterocycl. Chem.*, 3, 466 (1966).

86. Y. Makisumi and A. Murabayashi, *Tetrahedron Lett.*, 29, 2449 (1969).

87. M. Robba, J-M. Lecomte and M.C. de Sevricourt, *Bull. Soc. Chim. France*, 10, 3630 (1970).

88. M. Robba, J-M. Lecomte and M.C. de Servicourt, *C.R. Acad. Sci. Paris, Sec. C*, 266, 128 (1968); *Chem. Abstr.*, 69, 27365j (1968).

89. F. Sauter and W. Deinhammer, *Monat. Chem.*, 104, 101 (1973).

90. A. Zimmer, A. Prox, H. Pelzer and R. Hamkwitz, *Biochemical Pharmacology*, 22, 2213 (1973).

91. S.W. Schneller and F.W. Clough, *J. Org. Chem.*, in press.

92. W.F. Gray, I.R. Pomerantz and R.D. Ross, *J. Heterocycl. Chem.*, 9, 707 (1972).

93. A. Sakurai and M. Goto, *Tetrahedron Lett.*, 25, 2941 (1968).

94. R. Motoyama and E. Imoto, *Nippon Kagaku Zasshi*, 78, 793 (1957); *Chem. Abstr.*, 54, 22560e (1960).

95. S. Nishimura and E. Imoto, *Nippon Kagaku Zasshi*, 82, 1680 (1961); *Chem. Abstr.*, 59, 1619c (1963).

96. S. Nishimura, A. Sakumoto and E. Imoto, *Nippon Kagaku Zasshi*, 82, 1540 (1961); *Chem. Abstr.*, 57, 15051b (1962).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

97. L.H. Klemm, W.O. Johnson, and D.V. White, *J. Heterocycl. Chem.*, 9, 843 (1972).

98. J. Lice, *J. Org. Chem.*, 19, 70 (1954).

99. E.E. Campaigne, E.S. Neiss, and T.R. Bosin, *J. Med. Chem.*, 10, 270 (1967).

100. Reviews: F.D. Popp, *Advances in Heterocyclic Chemistry*, Vol. 9, Academic Press, New York, 1968; W.E. McEwen and L. Cobb, *Chem. Rev.*, 55, 511 (1955).

101. Preparations: F.D. Popp, W. Blount, and P. Melvin, *J. Org. Chem.*, 26, 4930 (1961); F.D. Popp and W. Blount, *ibid.*, 27, 297 (1962); F.D. Popp and A. Soto, *J. Chem. Soc.*, 1760 (1963).

102. M.P. Cava, M.V. Lakshmikantham and M.J. Mitchell, *J. Org. Chem.*, 34, 2665 (1969); F.D. Popp and W.E. McEwen, *J. Amer. Chem. Soc.*, 79, 3773 (1957).

103. F.D. Popp, *J. Heterocycl. Chem.*, 1, 107 (1964).

104. E.C. Taylor and S.F. Martin, *J. Amer. Chem. Soc.*, 96, 8095 (1974).

105. F. Eloy and A. Deryckere, *Bull. Chim. Therapeutique*, 6, 466 (1969).

106. K.W. Bentley, *The Alkaloids*, Vol. I, Interscience Publishers, New York, 1957.

107. S,W. Schneller and F.W. Clough, *J. Heterocycl. Chem.*, 11, 975 (1974).

108. A.A. Santilli, D.H. Kim and S.V. Wanser, *J. Heterocycl. Chem.*, 8, 445 (1971).

109. J.R. Beck, *J. Org. Chem.*, 37, 3224 (1972); J.R. Beck and Y.A. Yahner, *ibid.*, 38, 2450 (1973).

110. E.C. Taylor and A. Crovetti, *J. Org. Chem.*, 19, 1633 (1954).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

111. G.M. Coppola, G.E. Hardtmann, and B.S. Huegi, *J. Heterocycl. Chem.*, 11, 51 (1974).

112. E. Matsumura and M. Ariga, *Bull. Chem. Soc. Japan*, 46, 3144 (1973).

113. S. Raynolds and R. Levine, *J. Amer. Chem. Soc.*, 82, 472 (1960).

114. E.C. Taylor and A.J. Crovetti, *J. Org. Chem.*, 25 850 (1960).

115. R. Levine, M.J. Karten, and W.M. Kadunce, *J. Org. Chem.*, 40, 1770 (1975).

116. Von K. Gewald, M. Hentschel, and U. Illgen, *J. prakt. Chem.*, 316, S-1030 (1974); E. Koenigs and H. Geisler, *Chem. Ber.*, 57B, 2076 (1924); A.E. Tschitchibabin and N.N. Woroshtzow, *Chem. Ber.*, 66B, 364 (1933).

117. G.R. Clemo and H. Koenig, *J. Chem. Soc.*, S-231 (1949).

118. Ian Fleming, *Selected Organic Syntheses*, John Wiley and Sons, Inc., New York, 1973.

119. *The Chemistry of Heterocyclic Compounds*, Vol. 3, *Thiophene and Its Derivatives*, H.D. Hartough, Ed., Interscience Publishers, New York, 1952.

120. F.L. Muehlmann and A.R. Day, *J. Amer. Chem. Soc.*, 78, 242 (1956).

121. M. Asui, *Yakugaku Zasshi*, 81, 1475 (1961); *Chem. Abstr.*, 56, 8711h (1962).

122. O. Fuentes and W.W. Paudler, *J. Org. Chem.*, 40, 1210 (1975).

123. L.H. Klemm, R. Zell, I.T. Barnish, R.A. Klemm, C.E. Klopfenstein and D.R. McCoy, *J. Heterocycl. Chem.*, 7, 373 (1970).

124. J.A. Joule and G.F. Smith, *Heterocyclic Chemistry*, Van Nostrand Reinhold Co., New York, 1972.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

125. W.S. King and F.F. Nord, *J. Org. Chem.*, 14, 405 (1949).

126. T. Yabunchi, *Chem. Pharm. Bull.*, 8, 169 (1970).

127. F. Binns, J.A.G. King, A. Peraval, N.C. Robson and G.A. Swain, *J. Chem. Soc., C*, 1134 (1970).

128. T.C. Owen, *Characterization of Organic Compounds by Chemical Methods*, Marcel Dekker, Inc., New York, 1969.

129. S.W. Schneller and F.W. Clough, *J. Heterocycl. Chem.*, 12, 513 (1975).

130. S.W. Schneller and F.W. Clough, *Heterocycles*, 3, 135 (1975).

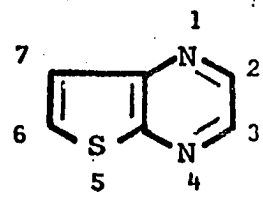
131. F.J. Tinney, Parke, Davis and Company, personal communication.

132. Graciously provided by S.W. Schneller.

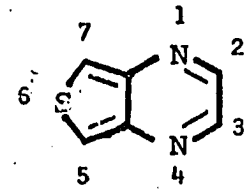
133. A. Helland and P.N. Skanke, *Acta. Chem. Scand.*, 26, 2601 (1972).

134. M.J. Haddadin, N.C. Chelhot, and M. Pieridou, *J. Org. Chem.*, 39, 3278 (1974).

135. The correct atom numbering and ring orientations for ring systems 86 and 87 are indicated below with structures i and ii. The alternatives (*i.e.* 86 and 87) employed herein are to simplify molecular orbital data comparisons with the related thienopyridine molecules.



i



ii

136. N. Rabjohn, Ed., *Organic Synthesis*, Coll. Vol. IV, p. 654, John Wiley and Sons, Inc., New York, 1963.

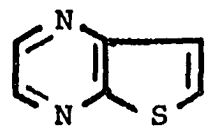
137. W. Herz and L. Tsai, *J. Amer. Chem. Soc.*, 76, 484 (1954).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

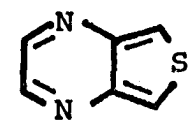
Appendix *

Molecular Orbital Studies of the Thienopyrazines

Until the recent disclosure[91] of the preparation of thieno[2,3-b]pyrazine (86), this heterocycle had been reported only as a constituent of polycyclic molecular arrays. In view of the isoelectronic and isosteric relationships between 86 and quinoxaline, a knowledge of the properties of 86 becomes quite significant to the direction and design of future studies involving 86. To this end, the molecular orbital calculations for 86 have been performed and are presented here. For comparative purposes, similar data for the unknown thieno[3,4-b]pyrazine (87) has also been determined and is discussed below. The methods employed for these calculations have been discussed in detail elsewhere.[133]



86



87

Reactivities. The ability of a molecule to undergo different types of chemical reactions which are dependent on the nature of the reacting agent, may be expressed by various reactivity indicies. Such easily attainable[133]

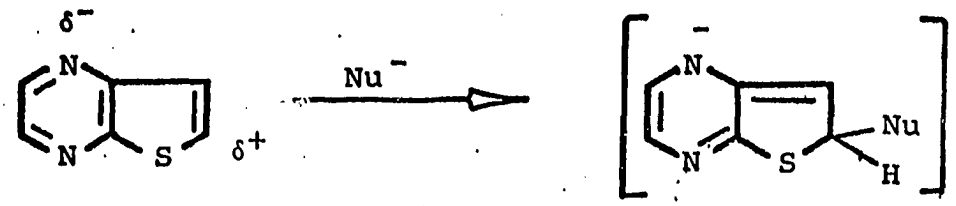
*The calculations presented herein were performed by Dr. Per Njal Skancke.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

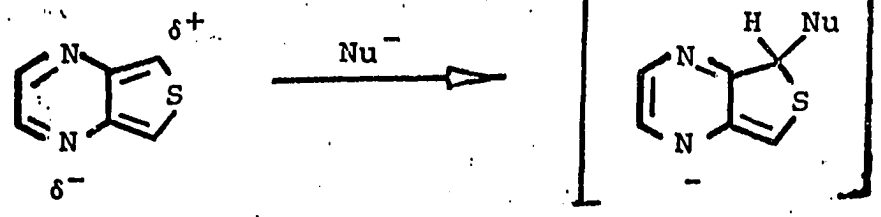
indicies are the frontier radical density (FRD), an index for radical substitution; the frontier orbital density (FOD), an index for nucleophilic substitution; and the frontier electron density (FED), an index for electrophilic substitution. These results for 86 and 87 are presented in Table 5.

As revealed by the FRD and FED indicies, both 86 and 87 should undergo free radical and electrophilic substitutions at C-3 in the former and C-1 and C-3 in the latter. This is not surprising in view of the calculated and experimental results which have been reported for related thieno[2,3-b]-, thieno[3,2-b]-, thieno[3,4-b]-, and thieno[3,4-c]pyridines. (See references 68, 97, 123, 133, and 64.) On the other hand, the FOD index indicates that nucleophilic substitution will occur at C-2 in 86 and C-1 and C-3 in 87. It is noteworthy that the carbons adjacent to the ring nitrogens have a lower FOD than those next to the sulfur atom. However, similar data has been accrued for thieno[2,3-b]- and thieno[3,2-b]pyridine[133] and may be rationalized as being due to the extended vinylogous relationships of C-2 in 86 and C-1 and C-3 in 87 with the pyrazine ring nitrogens. This, in turn, would imply that a polarized species (*i.e.* 186 and 187) is prevalent for 86 and 87 under nucleophilic reaction conditions and that H-2 in 86 and H-1/H-3 in 87 should be sufficiently acidic to react with an appropriate base.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



186



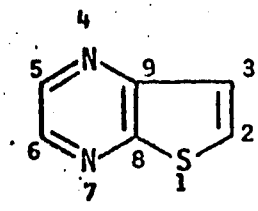
187

Bond Lengths. Table 6 presents the calculated bond distances for 86 and 87. It is obvious from this data that the bonding situation is close to that for the structurally related thienopyridines and suggests that a certain amount of bond fixation exists in the pyrazine portion of 86. A similar picture unfolds for the thienopyridines[133] and is certainly a manifestation of the electronic requirements imposed by a fused thiophene moiety. On the other hand, the bonding fixation situation for 87 is more pronounced, as with thieno[3,4-b]pyridine,[133] and indicates that the C-1 and C-3 of its thiophene portion should behave as the termini of a s-butadiene fragment. This expectation is corroborated by the reported[97] Diels-Alder reaction of thieno[3,4-b]-quinoxaline (90, page 59) with N-phenylmaleimide.

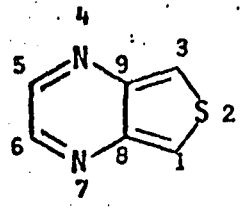
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 5

Reactivity Indicies for Compounds 86 and 87



86



87

Reactivity Indicies

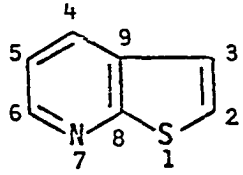
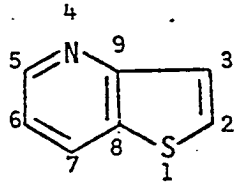
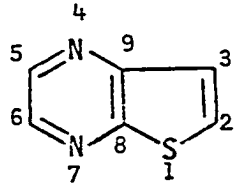
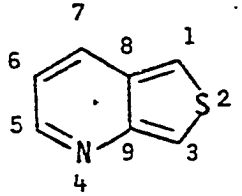
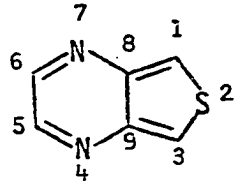
Molecule 86				Molecule 87			
Atom	FRD	FOD	FED	Atom	FRD	FOD	FED
2	0.208	0.387	0.028	1	0.382	0.281	0.482
3	0.261	0.281	0.240	3	0.382	0.281	0.482
4	0.194	0.280	0.108	4	0.281	0.291	0.271
5	0.041	---	0.081	5	0.152	0.177	0.127
6	0.164	0.305	0.022	6	0.152	0.177	0.127
7	0.149	0.177	0.120	7	0.281	0.291	0.271
8	0.029	0.058	---	8	0.028	0.024	0.032
9	0.125	0.229	0.021	9	0.028	0.024	0.032

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 6

Comparison of Calculated Bond Distances in Å for Thieno[2,3-b]- and Thieno[3,2-b]pyridine with Thieno[2,3-b]pyrazine and Thieno[3,4-b]pyridine with Thieno[3,4-b]pyrazine[135]

Bond					
1-8	1.727 Å	1.727 Å	1.727 Å	1.381 Å	1.384 Å
1-2	1.724	1.725	1.725	1.707	1.706
2-3	1.356	1.355	1.355	1.708	1.706
3-9	1.451	1.452	1.452	1.380	1.384
4-9	1.411	1.351	1.351	1.446	1.378
4-5	1.387	1.329	1.328	1.364	1.310
5-6	1.408	1.408	1.411	1.438	1.433
6-7	1.330	1.389	1.329	1.307	1.310
7-8	1.347	1.406	1.347	1.382	1.378
8-9	1.404	1.405	1.406	1.441	1.442