SYNTHESIS OF SELECTED HETEROCYCLES AND SPECTRAL ANALYSIS OF MOLECULAR COMPLEXES BETWEEN HETEROCYCLIC STEROIDAL SYSTEMS (AND MODEL COMPOUNDS) AND ANTICANCER AGENTS

By

GEORGE XAVIER THYVELIKAKATH

Bachelor of Science
University of Kerala
Kerala, India
1965

Master of Science
University of Southwestern Louisiana
Lafayette, Louisiana
1972

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1975

SYNTHESIS OF SELECTED HETEROCYCLES AND SPECTRAL ANALYSIS OF MOLECULAR COMPLEXES BETWEEN HETEROCYCLIC STEROIDAL SYSTEMS (AND MODEL COMPOUNDS) AND ANTICANCER AGENTS

Thesis Approved:

K D Berlin
Thesis Adviser

H. L. Seubert II

Ernest M. Hodnett

Norman N Durham

Norman N Durham
Dean of the Graduate College

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation and thanks to Dr. K. D. Berlin for furnishing the incentive, cooperation and excellent guidance necessary for this investigation. His faith, personal counsel and friendship throughout the course of this graduate study are gratefully acknowledged. I am grateful to Dr. O. C. Dermer for his advice concerning nomenclature and for proofreading this manuscript. Special thanks are extended to Dr. N. N. Durham for his guidance and contagious enthusiasm for research and to Dr. R. W. Chesnut in the Microbiology Department for preliminary microbial and tissue culture screening. Appreciation is also extended to Dr. D. Van der Helm for performing single-crystal x-ray analysis of several compounds in this study. I would also like to thank Dr. E. M. Hodnett and Dr. H. L. Gearhart for serving as committee members and their frequent direction in completing this investigation.

I am indebted to Mrs. Joyce Gazaway for the typing of this manuscript. The help of Mr. Stan Sigle in obtaining 100 MHz NMR spectra, Mr. Norman Perreira in obtaining mass spectral data, and Mr. G. Prakash in acquiring graphic mass spectral data is gratefully appreciated.

Special gratitude is expressed to the U. S. P. H. S., NIH (IN-91C institutional grant) and the National Cancer Institute for a Research Assistantship and to Dow Chemical Company and Gulf Oil Company for summer fellowships. I am also thankful for financial support for the work

which has been received from the Department of Chemistry in the form of teaching assistantships.

I am particularly indebted to my wife, Mary, for her encouragement, devotion, faith, and understanding, while providing a home free of tension and full of love and affection.

I am dedicating this thesis to my parents, Xavier and Annie, whose spiritual, material and inspirational encouragement and thorough understanding have made this goal possible.

The timely assistance, cooperation, and sincere friendship of the members of Dr. Berlin's research group during the course of my graduate career are gratefully remembered. To my fellow graduate students and the faculty of the Department of Chemistry, I extend thanks for help with problems during the course of this work. Finally, I wish to express my gratitude to many residents of Stillwater (especially Dr. and Mrs. Berlin and family) who have helped to make the last three and one-half years in this town a genuine educational experience. Above all, may I thank God for giving me this opportunity to fulfill His will successfully.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ILLUSTRATIONS

Infrared Spectra

CHAPTER I

HISTORICAL

Cancer Chemotherapy - General Background

In 1937, the National Cancer Institute Act[201] was signed by President Franklin D. Roosevelt to establish "in the public health service a division which shall be known as the National Cancer Institute . . ." in order to conduct ". . . studies relating to the cause, diagnosis and treatment of cancer." Thirty-eight years have elapsed since the act of 1937. Man has embarked on a great voyage of significant discoveries.

Cancer is a generic term used for a large number and wide variety of malignant neoplasms, affecting the different organs and systems of the body. The etiology of cancer in a very few cases such as exposure to carcinogenic hydrocarbons or to excessive radiation or to action of viruses is only somewhat understood. In spite of intensive and prodigious efforts by countless medical investigators throughout the world, the "real" cause of cancer in human remains an enigma. Since any of the human body tissues may be affected by cancer, types of malignancies at present can only be discussed in a very general way, as malignant neoplasms of: (1) buccal cavity and pharynx, (2) digestive organs and peritoneum, (3) respiratory system, (4) breast and genitourinary organs, (5) lymphatic and hematopoietic tissues, and (6) other and unspecified sites.

One of the most crucial deficiencies of present cancer therapy appears to be the difficulty of detecting tumors at the beginning of the growth, when therapy has a high degree of success. By the time symptoms of cancer are discerned, it has usually metastasized and cure becomes very unlikely.[177] The main cancer therapeutic modalities of current use are surgery, radiation therapy, chemotherapy, immunotherapy and/or a combination[32] of one of these with another.

The rapidly growing interest and activity in cancer chemotherapy parallels an energetic, world-wide investigation of the elucidation of the molecular basis of action of drugs which may affect, among other things, nucleic acid or portein synthesis with selectivity and specificity. The underlying anticipation is that such information may well be utilized in the design of new drugs or modification of old ones which are currently being used as anticancer drugs.

It is well known that usefulness of many drugs stems from the fact that the cells of some tumors grow and divide more rapidly than do cells of most normal tissues. Thus the drug kills tumor cells faster than normal cells.

Until the present century, most drugs were fortuitously discovered products of nature. Discovery and development of a new drug now usually evolves from a combination of systematic, planned experiments and accidental or unexpected observations. Only a handful of all the compounds for which activity in experimental animal systems have been claimed, have clinical utility. The current chemical status of cancer chemotherapy have been categorically summarized as (Tables I, II, III, IV, and V)[93,171,252] (1) alkylating agents, (2) antimetabolites, (3) mitotic

TABLE I

ALKYLATING AGENT[93,171]

Common Name	Disease Entity
Cyclophosphamide (Cytoxan)	Breast cancer, melanoma, lung cancer, Hodgkin's disease, lymphosarcoma, reticulum-cell sarcoma
Chlorambucil (Leukeran)	Hodgkin's disease, lymphosarcoma, reticulum-cell sarcoma, breast cancer
Busulfan	Chronic myelogenous leukemia
Melphalan	Multiple myeloma, breast cancer, ovarian carcinoma
Mechlorethamine (Nitrogen mustard)	Hodgkin's disease, lymphosarcoma, reticulum-cell sarcoma

TABLE II

ANTIMETABOLITES[93,171]

Common Name	Disease Entity
6-Azauridine triacetate	Choriocarcinoma and myosis fungoides
Cytosine arabinoside (Cytosar)	Acute leukemia (granulocytic and lymphocitic)
5- Fluorouracil	Breast cancer, colon cancer, ovarian cancer, stomach cancer
6-Mercaptopurine (Purinethol)	Acute leukemia (both cases), chronic leukemia (granulocytic), chorio-carcinoma
Methotrexate (Amethoptenn)	Lymphocytic acute leukemia, breast cancer, choriocarcinoma, head and neck cancer, testicular cancer
Thioguanine	Granulocytic and lymphocytic acute leukemia

TABLE III

MITOTIC INHIBITORS AND RANDOM SYNTHETICS[93,171]

Common Name	Disease Entity
TMCA (Deacetyl Colchicine L-tartrate)	Granulocytic acute leukemia, melanoma
Vinblastine sulfate (Velban)	Breast cancer, choriocarcinoma, Hodgkin's disease, lymphosarcoma
Vincristine sulfate (Oncovin)	Lymphocytic acute leukemia, Ewing's sarcoma, Hodgkin's disease, lymphosarcoma, neuroblastoma, reticulum-cell sarcoma, Wilm's tumor, rhabdomyosarcoma
Hydroxyurea (Hydrea)	Granulocytic chronic leukemia, melanoma
Methyl-GAG	Granulocytic acute leukemia
o,p'-DDD (1,1-Dichloro-2-(o-chlorophenyl)-2-(P-chlorophenyl)-ethane)	Adrenal cancer
Procarbazine	Hodgkin's disease
L-Asparaginase	Lymphocytic acute leukemia
Imidazolecarboxamide	Melanoma

TABLE IV

ANTIBIOTICS[93,171]

Common Name	Disease Entity
Bleomycin	Head and neck cancer, Hodgkin's disease lymphosarcoma
Daunomycin (Daunorubicin)	Granulocytic and lymphocytic acute leukemia
Mithramycin (Mithracin)	Testicular cancer
Mitomycin C	Osteogenic sarcoma
Streptozotocin	Pancreatic islet cell tumors
Actinomycin D (Dactinomycin)	Choriocarcinoma, Ewing's sarcoma, Wilm's tumor, testicular cancer, melanoma
Adriamycin	Ewing's sarcoma, Hodgkin's disease, lung cancer, lymphosarcoma, neuroblastoma, osteogenic sarcoma

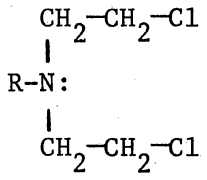
TABLE V

HORMONAL AGENTS[93,171]

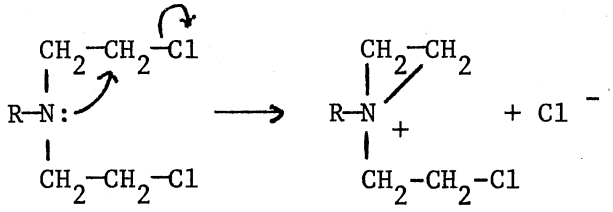
Common Name	Disease Entity
Cortisone	Lymphocytic acute leukemia, breast cancer, lymphosarcoma
Hydrocortisone	Lymphocytic acute leukemia, breast cancer, lymphosarcoma
Prednisolone	Lymphocytic acute leukemia, breast cancer, lymphosarcoma
Prednisone	Lymphocytic acute leukemia, lymphocytic chronic leukemia, breast cancer, lymphosarcoma, recticulum cell sarcoma
Delta-1-testololactone	Breast cancer
Fluoxymesterone	Breast cancer
Testosterone propionate	Breast cancer
Diethylstilbestrol	Breast cancer, prostatic cancer
Ethinylestradiol	Breast cancer, prostatic cancer
ACTH	Lymphocytic acute leukemia
Progesterone	Endometrial cancer

inhibitors, (4) antibiotics, (5) hormonal agents, and (6) random synthetics.

Many mechanisms have been proposed to explain the different stages of drug action.[171] But to date, no single mechanism can embrace all known observations and results for any of those drugs. There appears to be a common mechanism of action in drugs categorized under alkylating agents. Many of these drugs possess the general formula indicated below:[171]



Reaction may be with nucleophilic centers within the cell including a number of biologically important groups, e.g., phosphate, amino, sulfhydryl, hydroxyl, imidazole and carboxyl. This may take place through formation of the highly reactive, electrophilic ethylenimonium derivative from the teritary amine in neutral or alkaline aqueous solution according to the following general reaction:[171]



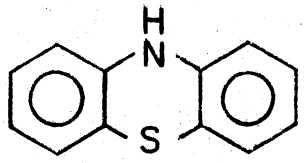
Efforts to identify metabolites responsible for the antitumor effects of many drugs are currently in progress. If successful, such data could lead to the ultimate development of anticancer agents with a high therapeutic index.

Undesirable side effects of drugs usually occur which may be attributed to cytotoxicity thereof.[93,171] Anorexia, nausea, vomiting, alopecia, dizziness, erythema, diarrhea, anemia, glossitis, leucopenia and thrombocytopenia are occasionally observed with some of the anti-cancer drugs.[93,171] Often on withdrawal of drugs the changes are reversible and the patients recover completely.[93,171] In some cases the changes may be accompanied by clinical illness.[252] A well known example is adriamycin, which has a broad spectrum of clinical activity.[252] Unfortunately, it can have a unique toxic effect and can cause damage to the heart with fatal cardiac failure. Obviously this situation reduces the length of time which the drug may be administered.

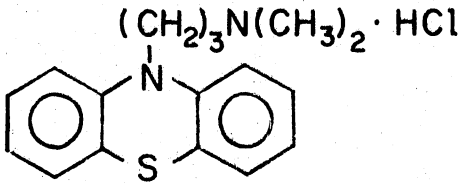
Heterosteroids and Model Systems

Biological activity of certain compounds may be ascribed to particular functionalities already built into the system. Some compounds containing a pyrazole,[109,176] a pyrazolone,[155,156] or an isoxazole[53,63,174,229] functionality (and certain derivatives[215,250] of urea and thiourea) have been found to be biologically active.

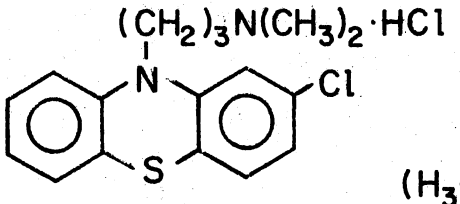
Since it has been reported[178] that specific enzymes are capable of using many drugs, pesticides, and other chemicals which contain sulfur as substrates, we considered it worthwhile to incorporate a sulfur atom into certain heterocyclic ring systems. Sulfur when present in certain heterocyclic ring systems is susceptible to enzymic oxidation to sulfoxide.[178] Phenothiazine (1) is oxidized[178] in this manner in the rat, as are the drugs promazine (2) and chlorpromazine (3). Methylene Blue (4) is not only partly converted to its sulfoxide in animal tissues but is also metabolized to the sulfone.[178]



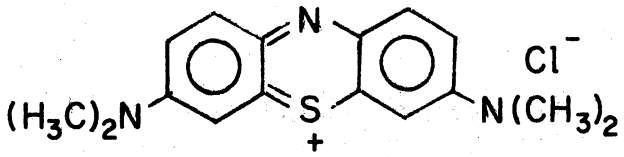
1



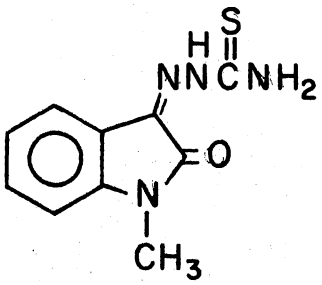
2



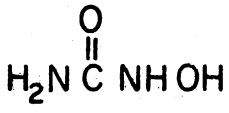
3



4



5



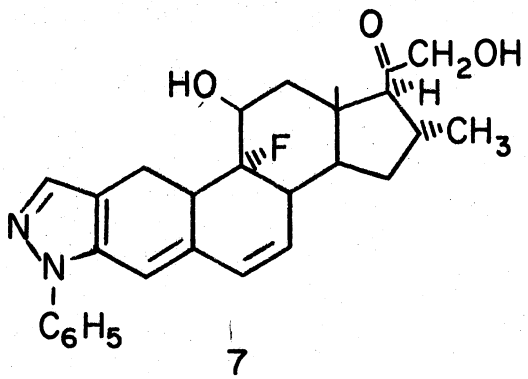
6

A number of organophosphorus compounds in common use as pesticides contain thioether groups.[122] In both plants and animals these chemicals are often susceptible to oxidation to the sulfoxide and sulfone.[122] Methisazone (5) is one of a group of derivatives of isatin-β-thiosemicarbazone that possesses antiviral activity.[250] Hydroxyurea (6) is an antitumor agent of simple structure that causes a relatively specific inhibition of DNA synthesis.[215]

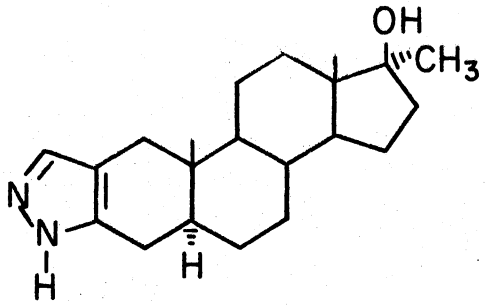
The [3,2-c]-2'-phenylpyrazole of 9α-fluoro-6-16α-dimethyl-Δ^6-hydrocortisone (7) is claimed to be a very potent antiinflammatory steroid known--over 2,000 times as powerful as hydrocortisone itself.[176] 17β-Hydroxy-17α-methylandrostano[3,2-c]pyrazole (8) possess a very favorable anabolic-to-androgenic activity ratio and has undergone clinical study.[2] 3β-Hydroxyandrostano[17,16-c]pyrazole (9) exhibits an antiovulatory activity one-fifth of that observed for norethisterone when administered orally in rats.[2] Sulfapyrazoles such as Orisul (10) showed a prolonged bacteriostatic action *in vivo*.[4,104,220]

A search for more effective pyrazolinones as drugs was stimulated by the discovery of 2,3-dimethyl-1-phenyl-3-pyrazolin-5-one by Knorr in 1883.[155] Pyrazolinones have been used in medicine as analgesics[156] and antipyretics.[156]

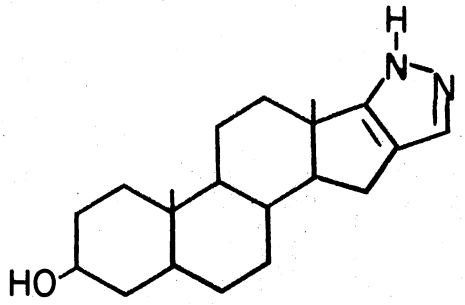
Medicinal applications of certain isoxazoles have been investigated since 1955 with the discovery of oxamycin.[118] Incorporation of an isoxazole functionality into sulfa drugs has produced strong activity *in vivo* against gram-positive and gram-negative bacteria.[229] 3,4-Dimethyl-5-sulfanilamidoisoxazole (Gantrisin) is an example in this class of drugs.[229] It has been proven that [2,3-d]isoxazole 11 shows 9.7 times as much anabolic activity as methyltestosterone, its precursor, while the androgenic activity was only 0.24 times.[63] The other isomer, [3,2-c]isoxazole 12, was found to be a strong anabolic agent. It is surprising that these compounds are devoid of estrogenic activity,[63] unlike the corresponding pyrazoles (compare with compound 8). Compounds 13 and 14, two isoxazolosteroids, have exhibited antitumor activity.[53]



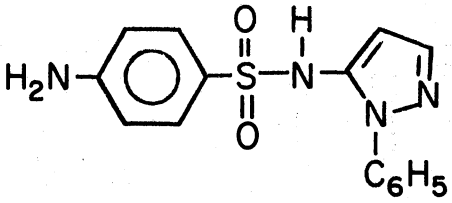
7



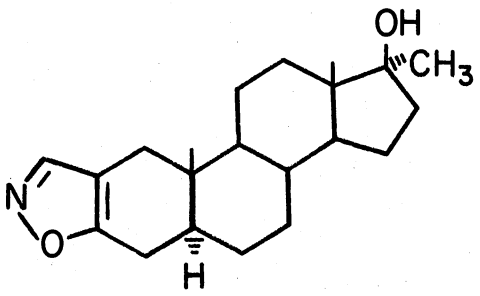
8



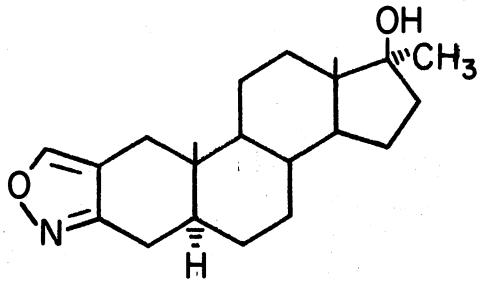
9



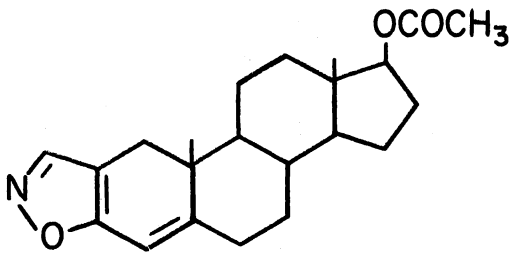
10



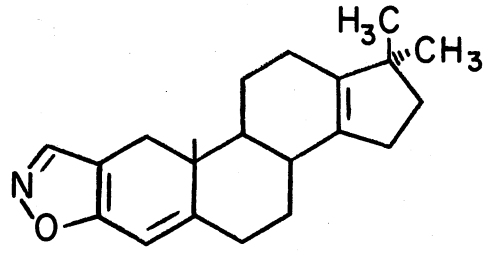
11



12



13



14

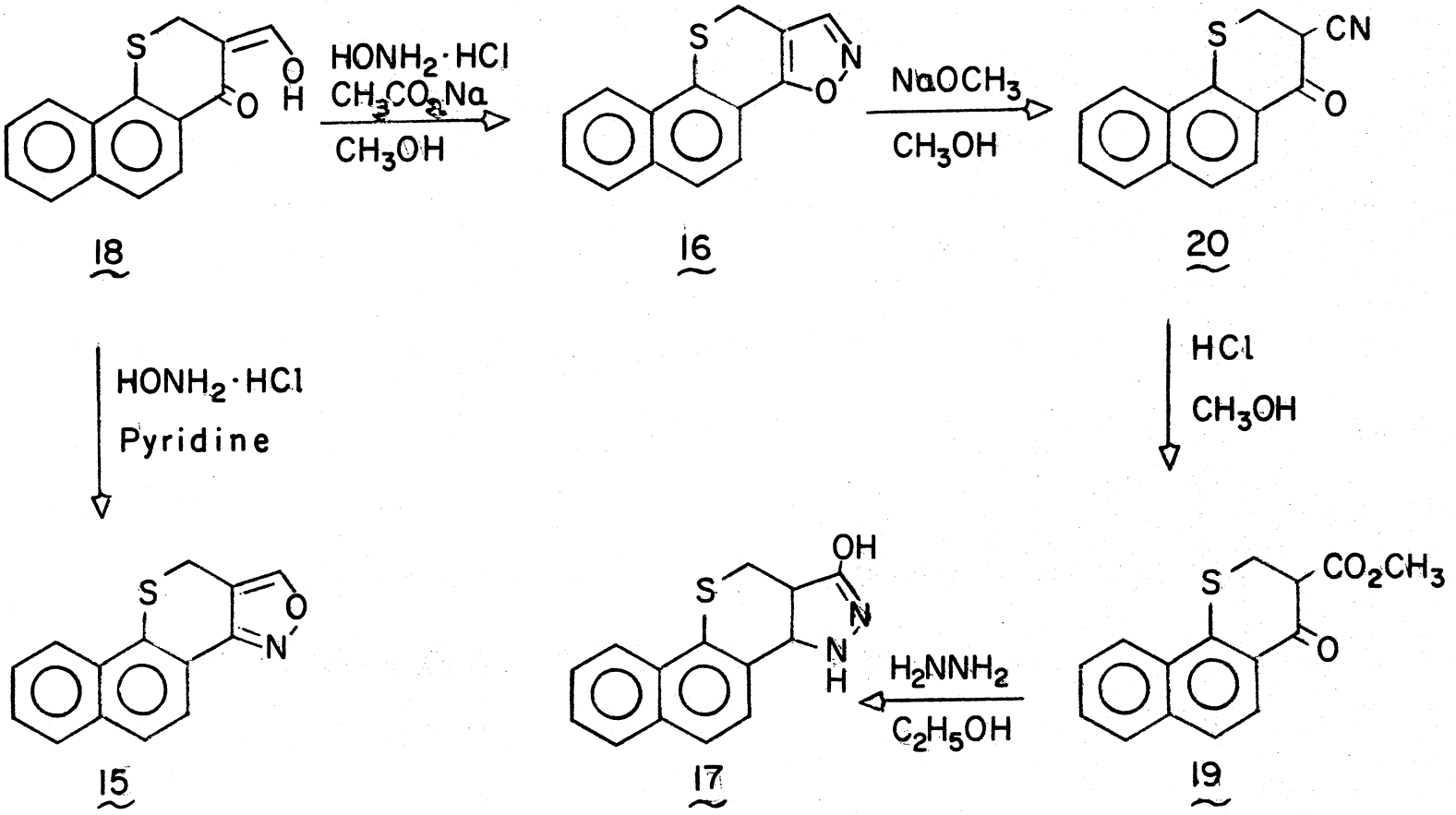
The chemistry and synthesis of pyrazoles and isoxazoles have been known to chemists since 1885. Both of these functionalities can easily be introduced to the corresponding 2-hydroxymethylene ketones. Benzo-[h]thiochromano[3,4-d]isoxazole (15), benzo[h]thiochromano[4,3-c]-isoxazole (16), and 1-hydroxy-3H-benzo[h]thiochromano[4,3-c]pyrazole (17) have been prepared from the 2-hydroxymethylene compound 18 (Scheme 1). 4-Methyl-7-methoxy-10,11-dihydro-2H-pyrazalo[3,4-i]-phenanthridine (21) was prepared from the corresponding α-hydroxy-methylene ketone 22 (Scheme 2).

Spectral studies of possible tautomer formation[5,22,39,42,43,44,45,50,52,62,90,97,99,105,106,113,135,137,159,169,185,186,248,249] in substituted and unsubstituted pyrazoles have been reported, and the structures were well characterized. A study of the spectra and acidity of unsubstituted pyrazoles and pyrazolones have been recently reported.[239]

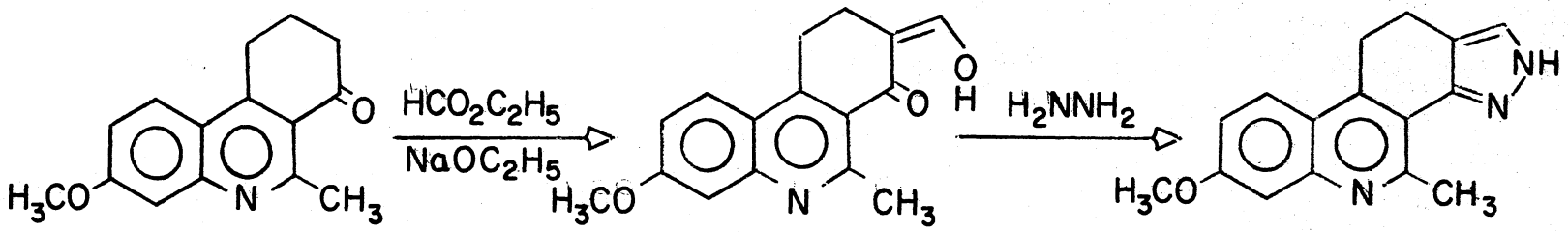
The ease with which isoxazoles can be synthesized is reflected in the large number of these compounds whose spectra have been analyzed.[29,30,147,148] Apart from simple structural analysis, NMR spectroscopy has been used to study isomers of isoxazoles.[138] Since controversy is still found in many publications[112,183] as to the common existence of two isomers (and in view of much NMR data supporting two structures), a critical ^{13}CMR analysis seems could very well solve this problem.

Molecular Complexation in Chemotherapy

The possibility of partial or complete reduction of side effects[72] of one drug when used in combination with others has prompted investigations of molecular complexes. It has also been found that uptake of


HONH2·HCl
CH3CO2Na
CH3OH
NaOCH3
CH3OH
HCl
CH3OH
HONH2·HCl
Pyridine
H2NNH2
C2H5OH
18
16
20
15
17
19

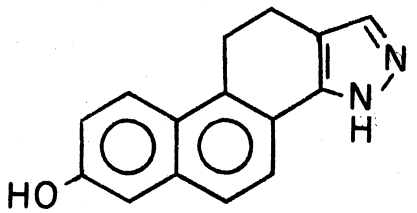
Scheme 1



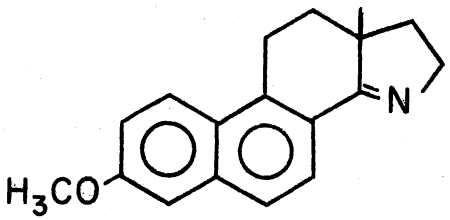
23 22 21

Scheme 2

nutrients by B. subtilis in the presence of actinomycin-D, a known anti-cancer drug, was greatly enhanced by the presence of an azasteroid.[58] It was concluded from spectral analysis that the potentiation of actinomycin D may be due to an azasteroid-actinomycin-D complex (Figure 1).[58] Results of the potentiation experiment using the hydroxy-pyrazole 24 in combination with actinomycin D against Pseudomonas fluorescens indicated that potentiation occurred and was quite pronounced (Figure 2).[119] The action of polymyxin, a surface-active antibiotic thought to cause disorganization of the cell membrane,[66] proved to be greatly potentiated when used in combination with the hydroxypyrazole 24 (Figure 3).[119] Potentiation of the polymyxin by the pyrazole 24 was observed using the gram-positive bacterium Bacillus subtilis W23.



24



25

Recently an NMR investigation has been extended to study complex formation[37] in vitro between vancomycin and acetyl-D-Ala-D-Ala. Perkins[211] has found that mucopeptide precursor molecules containing the terminal-D-Ala-D-Ala fragment bind strongly to the antibiotic vancomycin. This would be expected to interfere with bacterial cell-wall formation and offers a possible mechanism of action for the antibiotic. It was also found that the action of vancomycin was potentiated

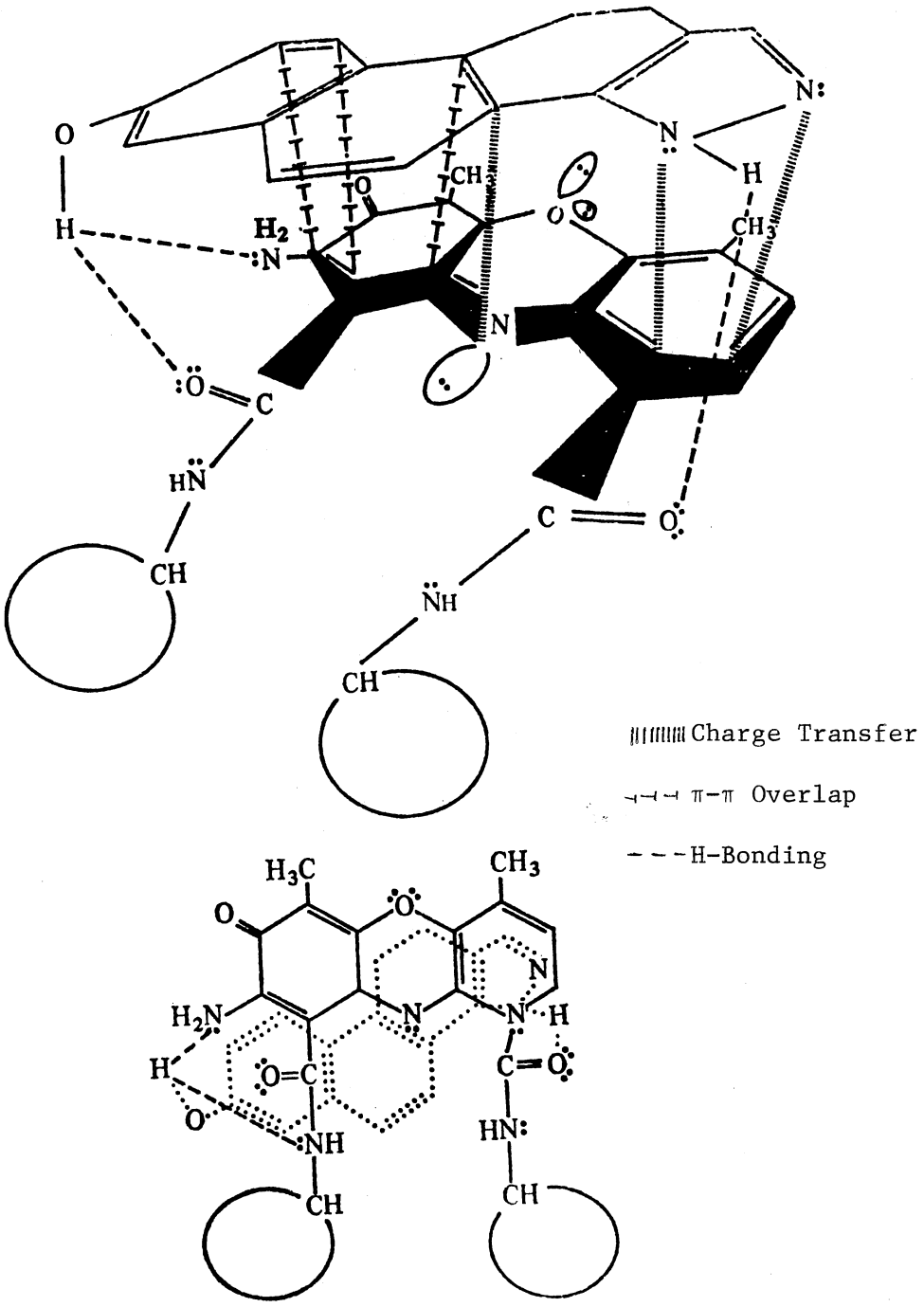


Figure 1. Proposed Configuration for the Actinomycin D-Indazole Complex

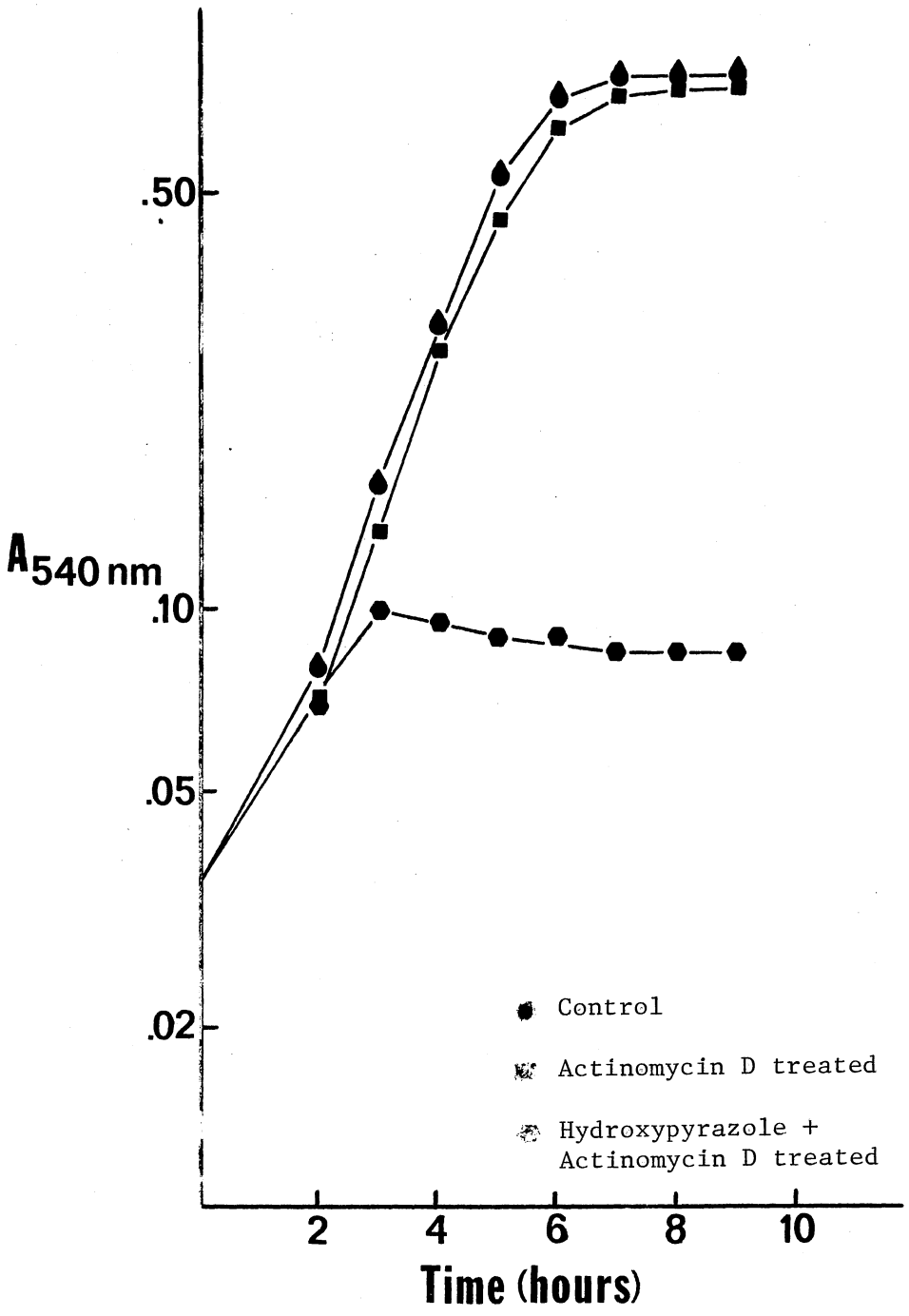


Figure 2. Hydroxypyrazole 24 Potentiation of Actinomycin D Action Against Pseudomonas fluorescens

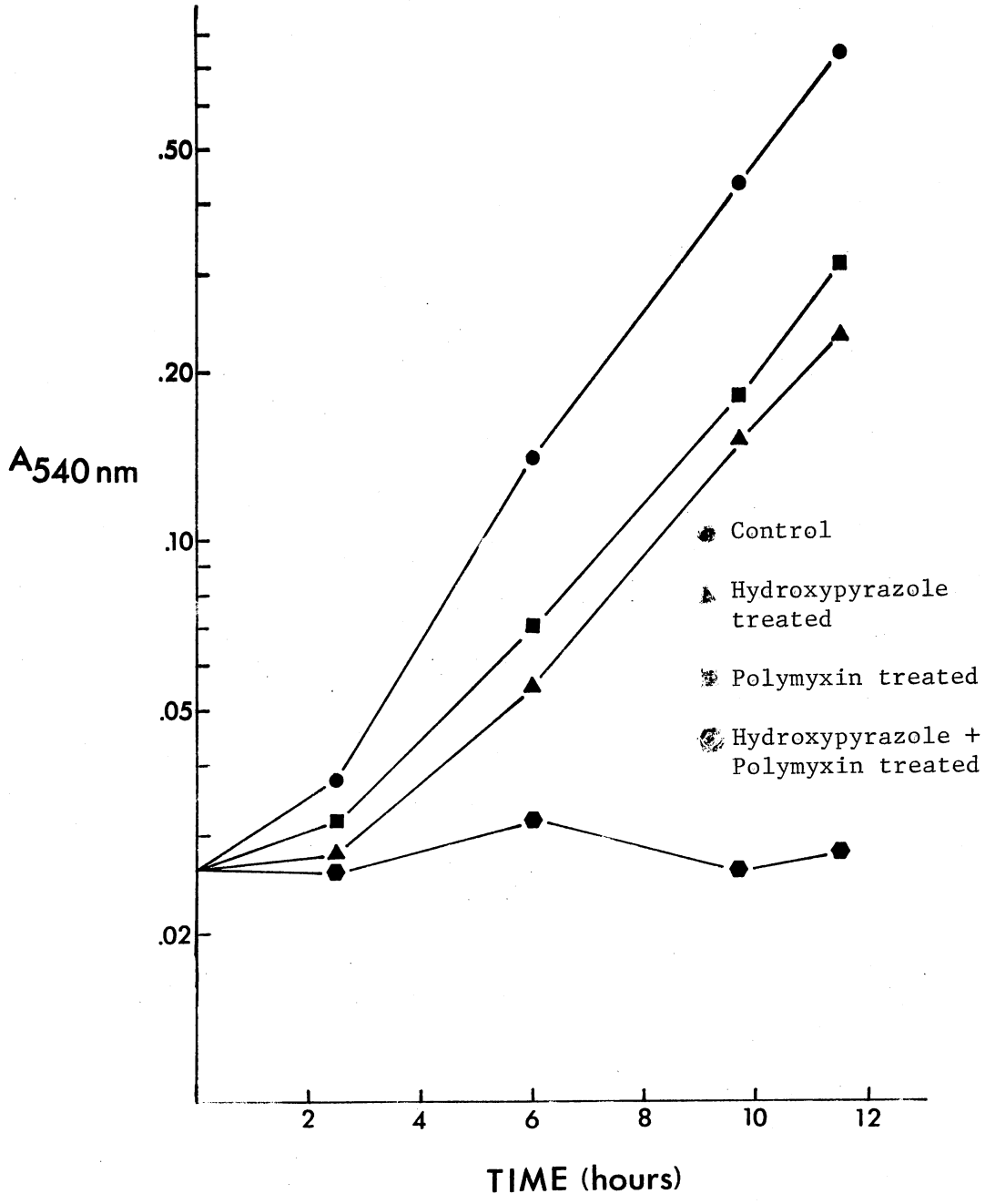
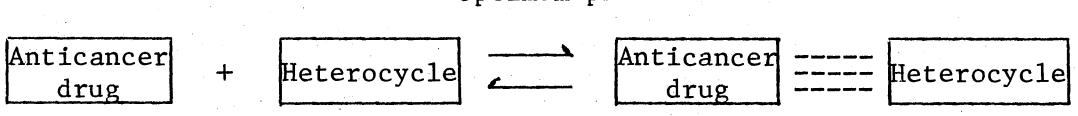


Figure 3. Hydroxypyrazole 24 Potentiation of Polymyxin Action Against B. subtilis W23

by the combination with methoxyimine 25 (Figure 4) against Bacillus subtilis.[59]

A primary goal of our research was to determine the nature of molecular complexes which are formed between selected anticancer drugs and certain heterocycles as part of a program to investigate the mode of action of heterocycles as potentiators of anticancer drugs, certain structural features in the molecules may be important. For example, the introduction of heteroatoms (like S, O, N, etc.) provides lone pairs of electrons for possible complexation sites (might act as charge transfer donor atoms) and for improved H-bonding potential.

A schematic representation of the general process of molecular complex formation may be envisioned as:



Since potentiators could act like donors, one might select an anticancer agent with "acceptor properties" for complexation studies. The use of the Pariser-Parr-Pople approximation,*[172] consideration of cost and easy availability of anticancer drugs, and finally reasons of solubility have prompted us to choose 5-fluorouracil as the acceptor candidate for complexation studies.

---

*The use of PPP approximation for π-electron density has led us to a major prediction concerning the ionization potentials of the nucleic bases. According to this approximation the increasing order of the π-ionization potentials should be in the following order for the heterocycles: Guanine < Adenine < Cytosine < Thymine < Uracil.

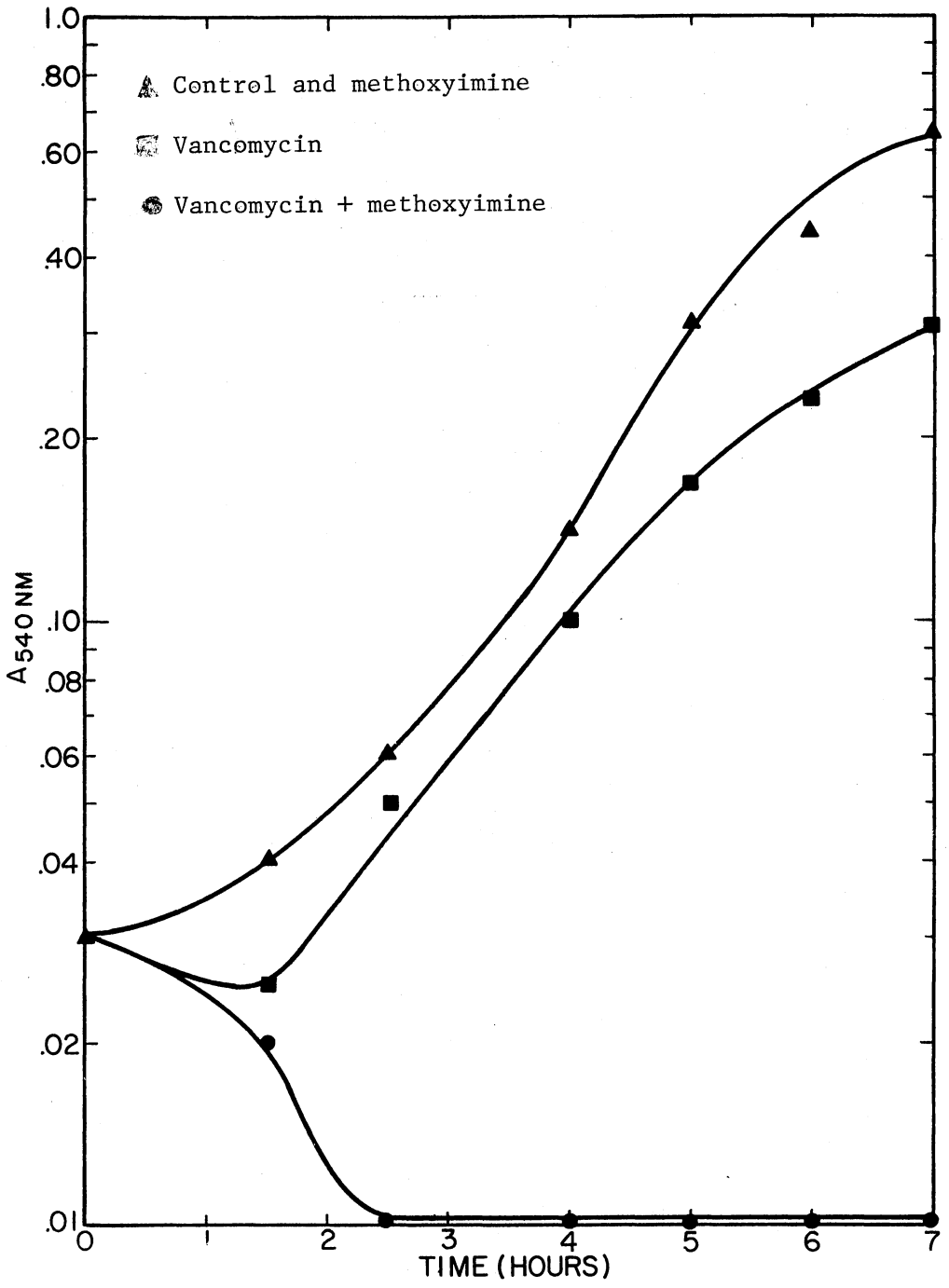


Figure 4. Potentiation of the Antibacterial Activity of Vancomycin Against B. subtilis by the Methoxyimine 25

Actually, the existence of intensely colored, molecular complexes has been known to chemists for many years.[197] Still the very nature of intermolecular bonds in molecular complexes is a matter of dispute.[110] But the tendency to accept the formation of weak complexes has been gaining momentum in recent years owing to the possible existence of such complexes in biological systems.[212]

As a first approximation, it may be logical to assume that the major contribution is displacement of electron density from the donor to the acceptor molecule.[110] But the low bond complex energies[46.49] in the π complexes have led to the suggestion that formation of very weakly bonded complexes may be due to van der Waals attractive forces, polarization forces, and dipole-dipole interactions and <u>not</u> necessarily to charge transfer.[110] Actually the extent of charge transfer, and hence the strength of binding between the components in the ground state, may be determined[13] by the ionization potential of the donor and the electron affinity of the acceptor.

The classification[195] of molecular complexes of the donor-acceptor type is based upon the type of orbitals involved in bond formation. Accordingly, the donor molecule may be divided structurally into three categories--η, σ and π and the acceptors into ν, σ and π. At least, nine different types of donor-acceptor complexes are possible. They are shown below with general examples in brackets.

(1) η-ν [$R_2O \cdot BX_3$]
(2) η-π [$R_2O \cdot Ar$]
(3) η-σ [$R_2O \cdot I_2$]
(4) π-ν [$Ar \cdot BX_3$]
(5) π-σ [$Ar \cdot I_2$]
(6) π-π [$Ar \cdot Ar$]
(7) σ-ν [$RX \cdot BX_3$]
(8) σ-σ [$RX \cdot I_2$]
(9) σ-π [$RX \cdot Ar$]

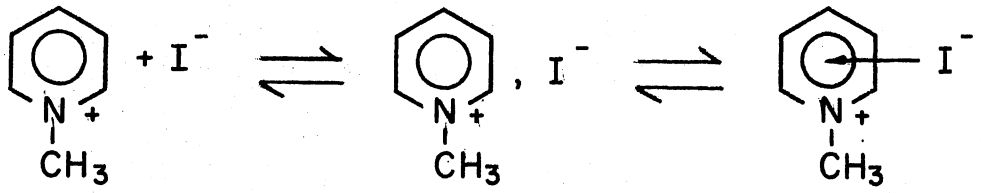
Among these, extensive investigations[204] have been made only with respect to complexes of type π-π and π-σ.

A number of general reviews[14,54,153,196,205,208] on charge-transfer complexes have been published as well as others more specifically concerned with energetics, spectra,[9,10,11,12,13,14] reactions[157] biochemical implications,[234] and many scattered papers with miscellaneous data.[61,65,70,71,83,104,128,129,130,131,132,145,154,160,162,163,164,207,230,233,240,241] The reader is referred to these for details of other properties of molecular complexes. Only very pertinent publications will be discussed here.

A donor-acceptor (DA) complex can also form by electrostatic attraction between an electron-rich donor (D) and electron-poor acceptor (A); the species can also be called a π complex.

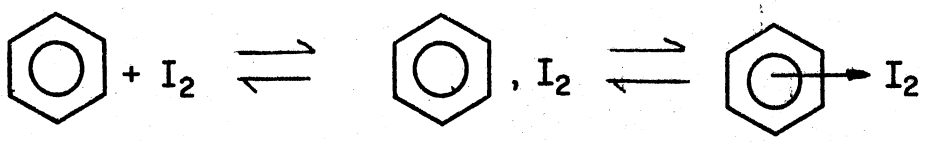
$$D + A \rightleftharpoons DA \rightleftharpoons D^{+}A^{-}$$

Additional stabilization of the complex can occur as a result of a minor amount of delocalization of one or more electrons over several atoms.[31,158] An example of a charge transfer is derived from N-methylpyridinium iodide (26); the electrostatic complex is depicted by (27) and the minor amount of charge transfer structure by (28).

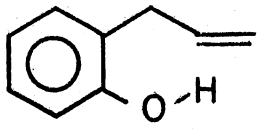


26 27 28

Another example of this type is benzene-iodine complex as pictured below. A very interesting example in this category is that involving



the π cloud of the vinyl group of o-allylphenol (29), which can act as an electron donor to the hydroxylic hydrogen as mentioned.[41]



29

The forces between an enzyme and an inhibitor or substrate that cause complex formation can be divided into two generalized classes:[41]

1. Complexes between electron donors and electron acceptors.

2. Nonpolar interactions of the van der Waals and hydrophobic bonding type.[41]

Usually there will be more than one interaction between an enzyme and its substrate.

Hydrogen bonding is perhaps one of the most significant secondary interactions that operates to alter the physical properties and/or maintain the integrity of specific configuration of biologically active compounds.[243] In biological systems as in others the most important hydrogen-bonding groups are OH and NH. Sulfhydryl groups do not often participate very effectively in hydrogen bonding, though they are capable of forming such bonds.[102] The possible donor groups on the enzyme for a hydrogen bond to an electron-deficient hydrogen on an inhibitor are[41] (a) the nitrogen lone electron pair on the imidazole ring of histidine, (b) the carboxylate anion of aspartate and glutamate,

(c) the amide carbonyl group of asparagine, glutamine, and the polyamide backbone, (d) the hydroxylic oxygen of serine, threonine, and tyrosine, (e) the lone sulfur electron pair of methionine and cystine, and (f) the π-electron system of the indole ring of tryptophan. The possible hydrogen acceptors on a protein[41] (a) the amide hydrogen of asparagine, glutamine, and the polyamide backbone, (b) the hydroxylic hydrogen of serine, threonine, and tyrosine, (c) the thiolic hydrogen of cystine, (d) the ring NH of tryptophan and histidine, and (e) possibly the ammonium group of arginine and lysine.

Although molecular complexes had long attracted theoretical speculations from chemists, real insight into their nature was only achieved by Brackman[33] and Mulliken[194] a little over a quarter-century ago. Much information on spectroscopic and thermodynamic behavior of charge-transfer complexes has been collected and discussed by Briegleb[48] and Andrews and Keefer.[13] The possible role of charge-transfer complexes in reactions has been examined carefully in only a few instances, even though such complexes are frequently postulated as intermediates.

Information about the equilibria between free reactants and π complexes and about the structure of π complexes, has come from many different experimental methods.[11,180] Useful approaches have included visible, ultraviolet,[180] and infrared spectroscopic techniques.[91] PMR analysis has been successfully used to evaluate equilibrium constants in hydrogen-bonding systems[26] and should be similarly useful in π-complex equilibria since the two types of equilibria are formally the same. Further, NMR analysis is extremely sensitive to small changes in the electronic environment of a magnetic nucleus.

When a small molecule is bound to a macromolecule, its rate of molecular motion, particularly rotational motion, is generally diminished, and restrictions of this nature will be revealed by increase in the "relaxation rates" of the protons in the bound molecules.[53a] It is probable that the rotational freedoms of the various parts of a bound molecule will not be affected to the same extent, the more tightly attached units being more restricted than those not so directly involved at the site of the binding.[53a] In such cases a selective change in correlation times can occur and can be detected by a selective broadening of the NMR signals in the spectrum of the bound molecule.[53a] It seems highly probable that a meaningful interpretation of relaxation rate measurements can reveal the nature of molecular interaction. The first application of these principles to be reported in full was a study of the binding of benzylpenicillin (penicillin G) to bovine serum albumin.[53a] This investigation[53a,100] illustrated well the techniques and safeguards in relaxation experiments and also showed how the results might be interpreted. (For details see reference 53a.)

A kinetic model was proposed for the exchange between free and bound molecules ($T_{exchange}$) as well as for the relaxation times.[53a,100] Three possible cases, depending on the rate of exchange between the states, can then be distinguished.[53a,100]

$$\frac{1}{T_{exchange}} < \left(\frac{1}{T_2}\right)_{free} < \left(\frac{1}{T_2}\right)_{bound} \qquad (1)$$

Rate: Very slow slow fast

or

$$\left(\frac{1}{T_2}\right)_{free} < \frac{1}{T_{exchange}} < \left(\frac{1}{T_2}\right)_{bound} \qquad (2)$$

Rate: Very slow slow fast

or

$$\left(\frac{1}{T_2}\right)_{free} < \left(\frac{1}{T_2}\right)_{bound} < \frac{1}{T_{exchange}} \qquad (3)$$

Rate: slow fast very fast

Equations (1) and (2) probably do not apply. If we assume equation (3) is valid, each relaxation process will have contributions from both free and bound species, and the time-averaged relaxation rate of the two forms may be given by:

$$\frac{1}{T_2} = B\left(\frac{1}{T_2}\right)_{bound} + (1 - B)\left(\frac{1}{T_2}\right)_{free} \qquad (4)$$

$$= \left(\frac{1}{T_2}\right)_{free} + B\left[\left(\frac{1}{T_2}\right)_{bound} - \left(\frac{1}{T_2}\right)_{free}\right] \qquad (5)$$

where B is the fraction of the total penicillin bound.

The problem is to obtain $\left(\frac{1}{T_2}\right)_{bound}$ experimentally. Let P and A be the total penicillin and albumin concentrations respectively, and assume that there are n noninteracting binding sites on each protein molecule. Then, on the basis of the law of mass action,

$$K = \frac{[\text{unbound penicillin}][\text{unbound sites}]}{PB}$$

$$K = \frac{P(1 - B)(nA - PB)}{PB} \qquad (6)$$

and on rearranging

$$P = \frac{K}{B - 1} + \frac{nA}{B} \tag{7}$$

To evaluate B [and hence $\left(\frac{1}{T_2}\right)_{bound}$ from equation (5)] and K, P and A must be plotted under conditions where B is a constant. P and A values which satisfy this requirement are established by using various known combinations of P and A concentrations.

Hammes and Tallman[114] used a treatment similar to that of Fischer and Jardetzky[100] for the calculations of binding constants from relaxation time data. The fraction of bound epinephrine, α, can be computed from the expression: (L-epinephrine-phosphatidylserine complex)

$$\alpha = \frac{\left(1/T_{2m}\right) - \left(1/T_{2f}\right)}{\left(1/T_{2b}\right) - \left(1/T_{2f}\right)} \tag{8}$$

where $\frac{1}{T_{2f}}$ is the reciprocal spin-spin relaxation time of the free epinephrine, $\frac{1}{T_{2b}}$ is the reciprocal relaxation time of the complexed epinephrine, and $\frac{1}{T_{2m}}$ is the reciprocal relaxation time of a mixture of epinephrine and phosphatidylserine of known composition. The value of $\frac{1}{T_{2f}}$ can be obtained by measuring the line width of the appropriate resonance signal of free epinephrine. The exact shape of this curve depends on the stoichiometry and stability of the complex formed. However, over the range of concentrations accessible in this study, the data appeared to approximate a straight line. The results of calculations of K, using different resonance signals and L-epinephrine

concentrations (and assuming a 1:1 interaction) are available.[114]

$$\text{L-epinephrine} + \text{phosphatidylserine} \overset{K}{\rightleftharpoons} \text{complex}$$

A most common approach for determination of equilibrium constants is that frequently referred to as the Benesi-Hildebrand method.[23,24] Consider the equilibrium

$$A + D \rightleftharpoons AD$$

where A and D represent acceptor and donor molecules, respectively, and AD represents the π-molecular complex. This method takes advantage of the fact that most π complexes have a new absorption band in the visible or ultraviolet region of the spectrum. From an appropriate plot of observed optical density versus donor concentration, the equilibrium quotient and molar absorbancy index of the complex can be calculated.

Hanna and Ashbaugh[117] derived an expression analogous to the Benesi-Hildebrand equation for use with NMR data. Consider the chemical shift of protons on A molecules which are undergoing rapid exchange[179] between the complexed and the free condition. Following treatments of data used in NMR studies of hydrogen-bonding equilibriums[21,136] it can be shown that

$$\left(\delta A_{obsd} - \delta A_o\right) = \frac{\xi_D Q}{1 + \xi_D Q}\left(\delta A_{AD} - \delta A_o\right) \qquad (9)$$

where δA_o is the shift of acceptor protons in uncomplexed form, δA_{obsd} is the observed shift of acceptor protons in complexing media, δA_{AD} is

the shift of the acceptor protons in the pure complex, and ξ_D is the concentration of donor on some arbitrary scale. Equation (9) requires that $\xi D >> \xi A$. It further assumes that the solutions are ideal, in which Q = K, or that the quotient $\frac{\gamma_{AD}}{\gamma_A \gamma_D}$ remains constant over the range of solutions studied. Defining $\Delta A_{obsd} = \delta A_{obsd} - \delta A_o$ and $\Delta A_{AD} = \delta A_{AD} - \delta A_o$, equation (9) becomes

$$\Delta A_{obsd} = \frac{\xi_D Q}{1 + \xi_D Q} \left(\Delta A_{AD}\right) \tag{10}$$

Writing equation (10) in reciprocal form gives

$$\frac{1}{\Delta A_{obsd}} = \frac{1}{Q \Delta A_{AD}} \frac{1}{\xi_D} + \frac{1}{\Delta A_{AD}} \tag{11}$$

Now this equation is analogous to the Benesi-Hildebrand equation except that the concentration of acceptor does not appear, and the shift of acceptor protons in pure complex replaces the molar absorbancy index of the complex. The first difference means that the chemical shift of acceptor protons does not depend on acceptor concentration as long as $\xi D >> \xi A$. This has been strongly supported in experiments on several different types of complexes.[115]

When we consider non-ideality of solutions, problem of activity coefficients appear to play a greater role. If we assume that on arbitrary concentration scale, the activity coefficient γ_A, γ_D, and γ_{AD} appropriate to this concentration scale of the species A, D, and AD, respectively, are such that the quotient $\frac{\gamma_{AD}}{\gamma_A \gamma_D}$ is not unity. The

equilibrium constant K may then be written:

$$K = \frac{[AD]}{[A][AD]} \cdot \frac{\gamma_{AD}}{\gamma_A \gamma_D}$$

This process further complicates the computation of association constants.

The primary question that arises is whether the equilibrium quotients derived from NMR data can be accepted with confidence or whether some type of solvent effect on spectra is important. This question is especially appropriate in view of the relatively small absolute value of the measured shifts. In order to explain these matters of extreme importance, two tables (Tables VI and VII)[94,116] are shown.

There are three factors[116] which argue that it is indeed the effect of molecular complexation that gives rise to the observed shifts. The first is that the size of the equilibrium constants are in the right order. That is, as the ionization potential of the donor becomes lower, the equilibrium constant increases.[116] The second is that a comparison with equilibrium quotients calculated from spectroscopic data[115] is possible and gives good agreement in some cases. The third is that, when the TCNQ* (an excellent receptor) is replaced by an aromatic molecule which is not expected[116] to complex with a weak donor, toluene for example, no shift of the protons on this molecule is observed under specified conditions. All of these facts argue strongly against the observed shifts of the TCNQ protons being due to some kind of a general

---

*7,7,8,8-Tetracyanoquinodimethane

TABLE VI

MEASURED AND CALCULATED PROPERTIES OF π-MOLECULAR COMPLEXES OF TCNQ AND A SERIES OF AROMATIC DONORS IN DIOXANE[116]

	Range of donor concentration, m	Max. $\Delta_{obsd}A$, c.p.s.	Q_m, kg. of solvent/ mole	$(\Delta_{AD}A)_m$, p.p.m.	Q_x, m.f.$^{-1}$	$(\Delta_{AD}A)_x$, p.p.m.
Benzene	0.468-2.15	8.6	0.061	1.28	---	---
Toluene	0.401-2.88	12.4	0.085	1.06	---	---
o-Xylene	0.353-2.54	13.7	0.12	0.91	0.47	2.86
Mesitylene	0.298-2.18	12.7	0.16	0.80	1.10	1.43
Durene	0.207-0.967	9.3	0.33	0.67	2.7	1.10
Pentamethylbenzene	0.056-0.457	8.0	0.55	0.59	6.2	0.67
Hexamethylbenzene	0.100-0.295	7.1	1.15	0.56	9.7	0.57

TABLE VII

THE MAXIMUM OBSERVED SHIFTS Δ_{max}, THE CALCULATED SHIFTS FOR THE PURE COMPLEX Δ_0 (BOTH RELATIVE TO 1,3,5-TRINITROBENZENE), AND THE ASSOCIATION CONSTANTS OF THE COMPLEX 1,3,5-TRINITROBENZENE + N,N-DIMETHYLANILINE IN VARIOUS SOLVENTS AT 33.5°

Solvent	Δ_{max} c/sec	Δ_0 c/sec	K kg/mole	K 1./mole
CCl_4	42.0	61.5	3.26	2.04
$HCCl_3$	45.5	76.5	0.72	0.45
CH_2Cl_2	37.5	80.4	0.39	0.25

solvent effect but argue for formation of a molecular complex.

Still we have to account for other factors. As evidenced from Table VI, the calculated equilibrium quotients and shifts of acceptor protons in the pure complex are dependent on the concentrations used. Since the quantity ΔA_{AD} is a function only of the structure of complex, it should be independent of the concentration scale. In fact, the values of ΔA_{AD} determined on the model scale are different from the corresponding quantities determined using the mole fraction scale. A small shift difference of the order of ≈ 1.0 Hz is understandable; but no good explanation for this phenomenon has been put forth.

In Table VII we observe that K values for charge-transfer complexes are very solvent-dependent. Thus, K for the complex between 1,3,5-trinitrobenzene and N,N-dimethylaniline is 20 times as great in CCl_4 and 60 times as great in cyclohexane as in dioxane. Interestingly, an optical determination of the equilibrium constant of the system N,N-dimethylaniline and 1,3,5-trinitrobenzene in CCl_4 gave K = 2.2 ℓ/mole, at 33.5°C, which is in good agreement with the NMR determined value, K = 2.04 ℓ/mole. Unfortunately, because of solubility limitations in many cases, it is very difficult to compare K values determined in different methods.

The ideal system for NMR study of molecular complexes would appear to be the following:

(1) Both donor and acceptor molecules should contain protons (or other magnetic nuclei), preferably giving a single sharp line.

(2) Equivalent donor and acceptor concentrations should be possible in a common solvent.

(3) The NMR absorptions of donor or solvent should not overlap with the absorption of acceptor (vice versa if donor shifts are being studied).

Unfortunately it is very difficult to find an experimental system for which these conditions all hold. Although a new technique, ^{13}CMR, offers several advantages, the need for large amounts of materials or else the cost of making ^{13}C enriched compound has caused progress to be slow.

Since in our investigation sulfur compounds have been used as donor candidates, thermodynamic and spectral properties of some sulfur compounds are given in Table VIII[16,161,189,202] for the sake of comparison. It is evident from the data that symmetry and overlap of the donor and acceptor orbitals appear far to outweigh the simple criterion of the electron affinity of the acceptor.[190] (This again complicates the molecular complexation studies.) Iodine has a lower electron affinity than TCNE (tetracyaoneothylene), but the spatial disposition of its σ_u antibonding orbital is far more suitable for complexation than the corresponding delocalized π-orbital of TCNE.[190]

The donor properties of sulfur, selenium, tellurium, and oxygen have been investigated.[79] The outer, lone-pair, p-orbitals of these atoms can be compared on the basis of size, and electron-donating ability as measured by gaseous ionization potentials.[79] The values of these parameters are listed in Table IX.[79]

The majority of these complexes involving sulfur compounds are not sufficiently stable to be isolated as solids. The heats of association are generally less than -10 Kcal/mole.[190]

TABLE VIII

THERMODYNAMIC AND SPECTRAL PROPERTIES OF MOLECULAR COMPLEXES OF AROMATIC AND HETEROCYCLIC SULFUR COMPOUNDS AT 25°C[16,161,189,202]

Donor	Acceptor	K_c (ℓ/mole)	$-\Delta H_c$ (kcal/mole)	Solvent
Thiophene	I_2		0.4	octane
Thianthrene	I_2	1.2	3.8	CCl_4
Phenoxanthiin	I_2	0.85	4.1	CCl_4
$(C_6H_5)_2S$	I_2		0.3	octane
$(C_6H_5)_2S_2$	TCNE	1.5		CH_2Cl_2
$(C_6H_5CH_2)_2S_2$	I_2	601	4.8	octane
$(C_6H_5CH_2)_2S_2$	TCNE	2.6		CH_2Cl_2
$(C_6H_5CH_2)_2S_2$	I_2		4.8	octane
$C_6H_5SCH_3$	I_2	9.2	6.1	CCl_4

TABLE IX

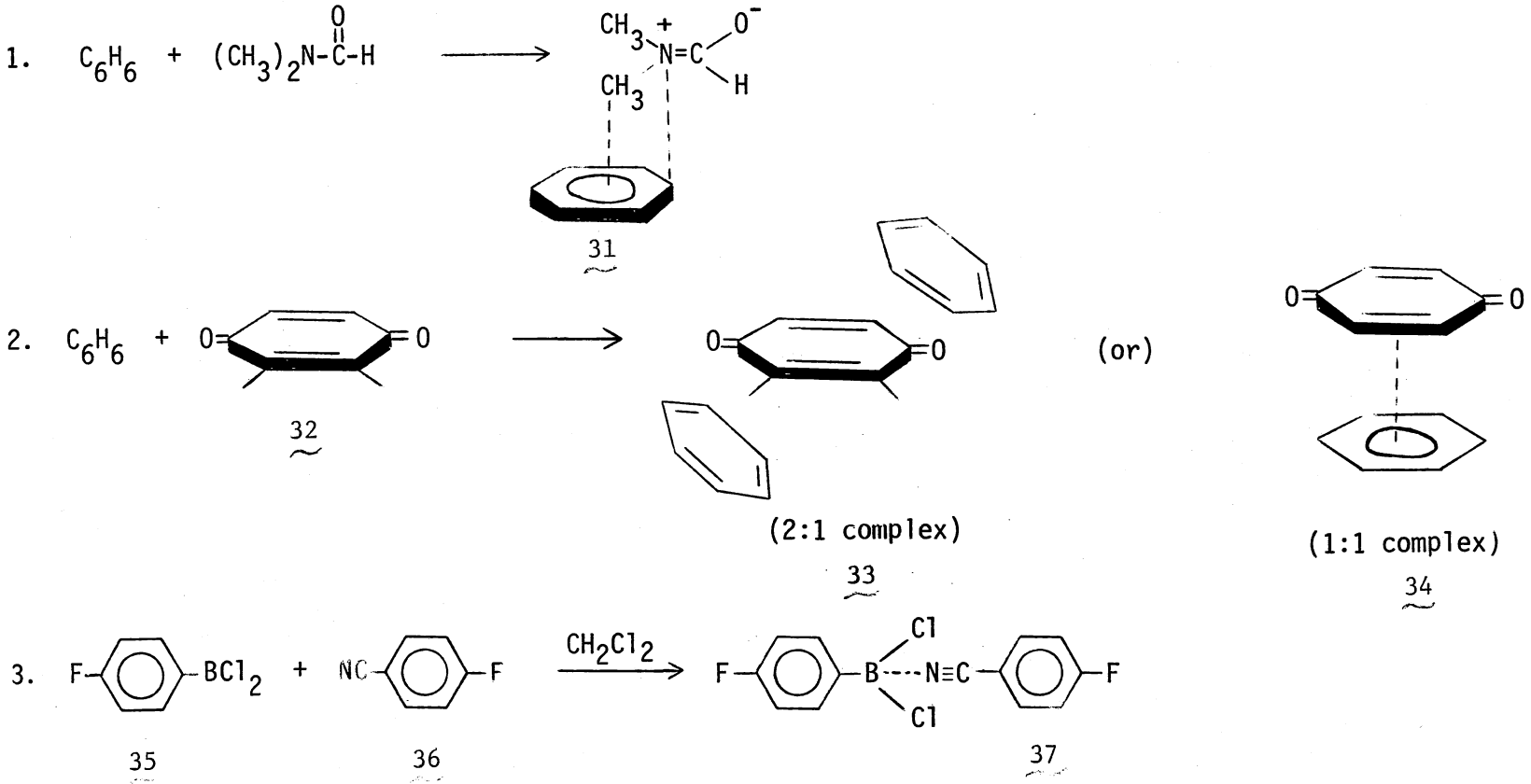
ATOMIC RADII AND IONIZATION POTENTIALS OF GROUP VI ATOMS[79]

Atom	Atomic Radius (A°)	Ionization Potentials (ev)
O	0.66	13.6
S	1.04	10.4
Se	1.17	9.8
Te	1.37	9.0

Scheme 3 shows three of the many reported cases[120,121,199,235] of molecular complex formation in solution. The proposed structures of these molecular complexes were deduced from NMR spectral analysis of the complexes and of the corresponding unbound candidates. Hatton and Richards[120,121] observed that the dilution of dimethylformamide with benzene caused a large upfield shift of the methyl proton resonance trans to the carbonyl function. It was further postulated that the association was between the electron-deficient nitrogen atom and the π-electrons in benzene. The oxygen atom comprising the negative end of the amide dipole is oriented as far away from the benzene ring as possible.

Laszalo and Williams[165] have measured the relative solvent shifts of methyl and methoxy protons in substituted p-benzoquinones and the existence of the complexes 33 and 34 have been postulated.[225] Complex 37 is a very good example for donor-acceptor type interaction. p-Fluorophenylborondichloride (35) acts like an acceptor and p-fluorobenzonitrile (36) acts like a donor candidate.

A variety of physical methods, other than already discussed, has been used for the experimental evaluation of K. They include: partition of one component of the complex between two immiscible solvents,[7.187] solubility,[8,9,10,35] and vapor pressure measurements including gas liquid chromatography,[68,69,74,75,76,103,209] polarography,[210,228] and kinetic measurements of the further irreversible chemical reactions of the components and/or the charge-transfer complex.[27,67,182] They also have involved calorimetry,[12,28] ultraviolet and visible spectroscopy,[80,84,95,96,152,170,200,226,244] and intensity measurements of infrared absorption bands,[144,166,184,188,216] and of


1. C_6H_6 + $(CH_3)_2N-C(=O)-H$
CH_3 + $N=C$ O^- H CH_3
31
2. C_6H_6 + O O
32
O O
(2:1 complex)
33
(or)
O O
(1:1 complex)
34
3. F BCl_2 + NC F
35
36
CH_2Cl_2
F B Cl Cl N≡C F
37

Scheme 3

Raman bands. It is not possible to review here the various techniques involved in these approaches or to detail the results which have been obtained.

The inability to produce satisfactory results using single drugs in patients with advanced cancer naturally led to the use of drug combinations.[81] Though theories[140] have been put forward to explain combination therapy, the mechanism of action of "drugs in combination" has not been totally viewed to include complexation in all cases prior to or in course of drug metabolism. It is not yet clear whether weak dipole-dipole interaction, or hydrophobic interactions, or even van der Waals forces produce synergism or antagonism. The classic example of what appears to be true synergism is the combined use of penicillin and streptomycin in the treatment of endocarditis due to Streptococcus faecalis.

One of three possible effects can be expected when drugs are used in combination: (1) drug indifference--that is, a result readily accounted for by the sum; (2) antagonism--a result less than the sum; and (3) synergism--a result greater than expected from the sum of activities of the individual agents. In general, a combination of bacteriostatic drugs is additive; a bactericidal plus a bacteriostatic drug may be antagonistic; and a bactericidal plus a bactericidal drug may show synergism.[140]

The use of combination drug therapy for cancer is gaining increasing acceptance. This has resulted from the demonstrated effectiveness of multiple-drug regimens in the treatment of acute leukemia,[101,131] Hodgkin's disease[82] and carcinoma of the breast.[51] Unfortunately, there are few guidelines that provide a rational basis for the selection

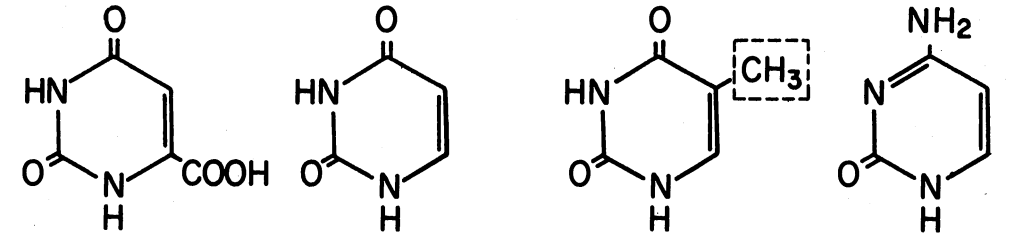
of agents to be used in combination, since little is known of the factors responsible for the augmented cytotoxic effects observed when certain drugs are administered together.

The combination of 5-fluorouracil (5-FU) with BCNU has been proved beneficial; patients do not experience diarrhea or mucocutaneous reactions as they do with 5-FU.[223] Nausea and vomiting were experienced with approximately equal frequency with all the regimens except mitomycin C alone which showed a slight advantage.[223] Diarrhea and mucocutaneous reactions were prominent in the 5-FU group, but were nonexistent with other single drugs and negligible with the drug combination.[223] It seems logical that proper selection of combination of drugs might reduce side effects of some anticancer agents.

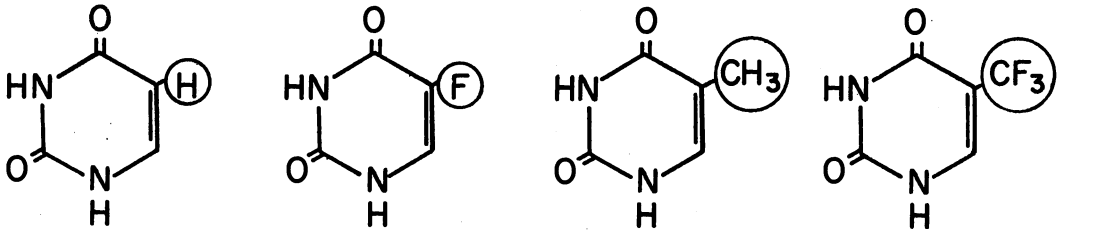
Since 5-fluorouracil has been selected as a major acceptor candidate for molecular complexation studies in our work, the chemistry and biological activity of this anticancer drug are given in the following section.

Orotic acid (38) has been considered the precursor of the nucleic acid pyrimidines. It was found in several transplanted tumors that uracil (39) was incorporated into DNA to a greater extent than was orotic acid.[126] The structures of the important natural pyrimidines are shown in Figure 5. Uracil is found in RNA and not in DNA, and thymine (40) is present in DNA and not RNA.[167] Moreover, it is well known that thymine, an essential building block of DNA, is made by the attachment of the methyl group to the ring system of uracil at the 5-position.

It was logical to assume that the strategic substitution of a fluorine atom for a hydrogen atom attached to C(5) might produce a



38 39 40 41



39 42 40 43

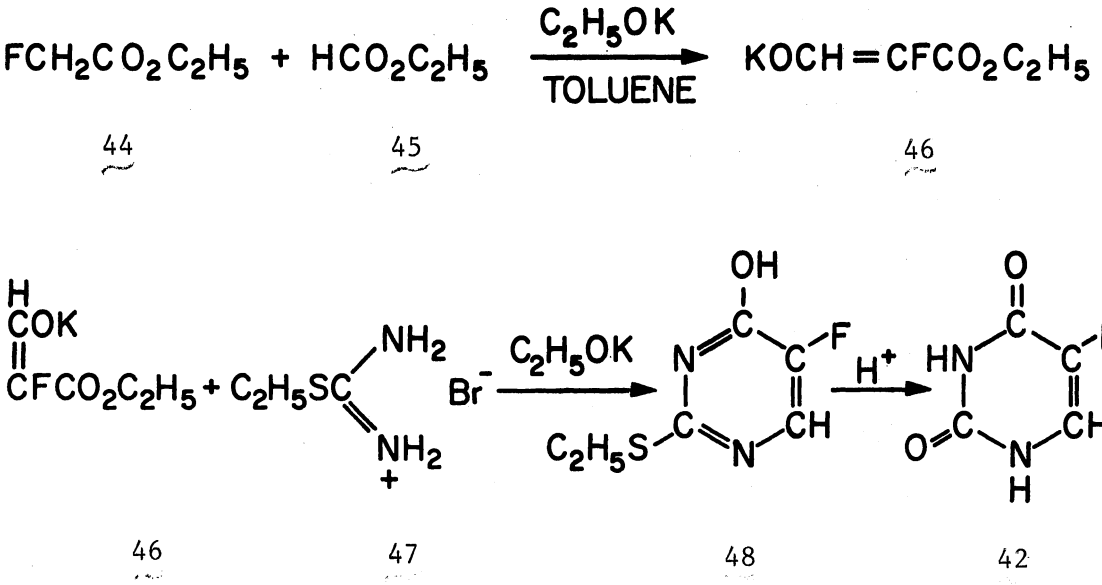
Figure 5. Nucleic Acid Pyrimidines and Some Model Compounds

significant change in biological effects. For example, such a substitution converts acetic acid to fluoroacetic acid and thus changes salad dressing to a commercial rat poison.

It was predicted that if 5-fluorouracil were to have biological activity it should block the attachment of the methyl group to the ring system of uracil, and hence block DNA synthesis. In addition, a fluorine atom is very similar in size to the hydrogen atom, and so it seemed that 5-fluorouracil should also be incorporated into RNA. The schematic representation of the synthesis of 5-fluorouracil is shown in Scheme 4.[89] The substitution of a hydrogen atom by a very strong electronegative fluorine atom also increased considerably the acid strength (pK_a = 8.0) compared to that of uracil (pK_a = 9.5).[25]

The first preliminary clinical report on 5-FU appeared in 1957 and initial clinical investigations were conducted at the University of Wisconsin Hospitals by Drs. F. J. Ansfield and A. R. Curreri.[73] They reported that 5-fluorouracil had profound activity in producing objective responses in patients suffering from disseminated breast and colon cancers, and this has been amply confirmed in many other clinics. After 10 years of experience, Ansfield and co-workers[15,123] clearly showed that the survival of recurrent breast cancer patients was significantly increased.

It is now clear from the work of Champe and Benzer[55] and Rosen and co-workers[227] that the presence of 5-FU in m-RNA can lead to a low frequency of translational errors as a consequence of the base-pairing of 5-fluorouracil with guanine as if it were cytosine (41). The biochemistry of the fluorinated pyrimidines is given in considerable detail in a review which appeared in 1965.[127]

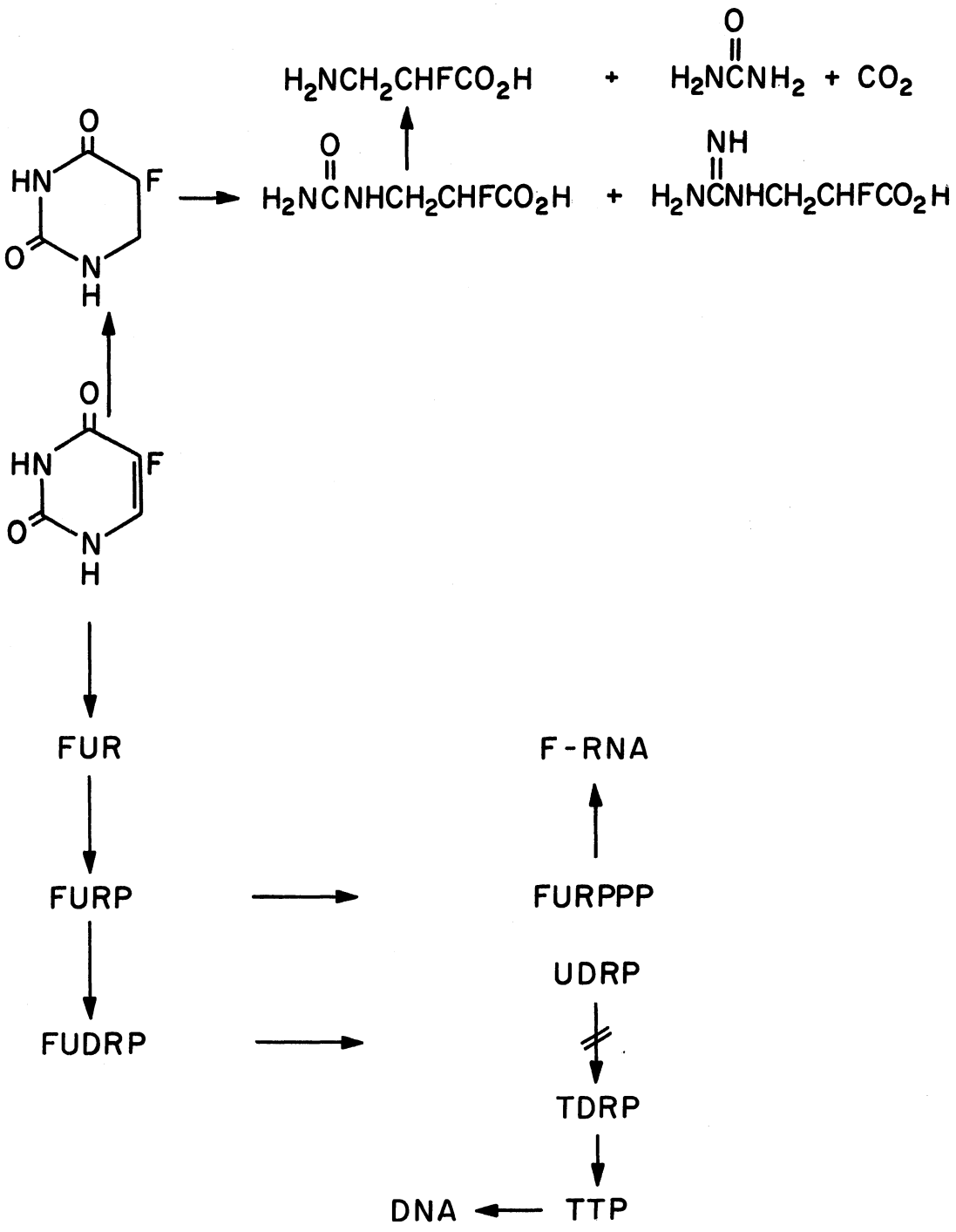

FCH2CO2C2H5 + HCO2C2H5
C2H5OK
TOLUENE
KOCH=CFCO2C2H5
44
45
46
H
COK
CFCO2C2H5 + C2H5SC
NH2
NH2
+
Br-
C2H5OK
OH
F
N
C
CH
C2H5S
N
H+
O
HN
F
O
N
H
46
47
48
42

Scheme 4

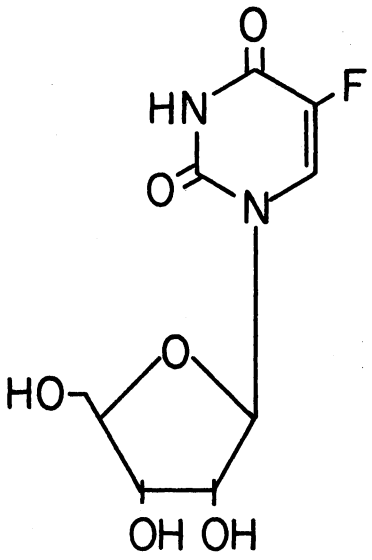
The metabolism[56,57,193] of 5-fluorouracil has been well characterized since 1960 (Scheme 5). When 5-fluorouracil was labeled with radiocarbon and administered to a few cancer patients, the 2-carbon atom quickly appeared in the respiratory carbon dioxide and was also excreted in the urine.[56] This led to a study of the degradation of 5-fluorouracil-2-C^{14} in mice and men, and it was demonstrated that the compound was degraded in a fashion exactly similar to that of uracil.[57] Since carbon dioxide and urea are the end-products of the catabolism of 5-FU-2-C^{14},[57] it was necessary to prepare 5-FU-6-C^{14} in order to determine the metabolic fate of the other moiety of the molecule. This was done, and it was shown that the catabolism of 5-fluorouracil takes place according to Scheme 5. The drug is first reduced systematically to dihydrofluorouracil, then hydrolyzed to α-fluoro-β-ureidopropionic acid (FUPA), which is converted to α-fluoro-β-guanidopropionic acid (FGPA), and then cleaved to α-fluoro-β-alanine(FBAL) and urea or carbon dioxide and ammonia.

Meanwhile, a series of nucleoside derivatives were prepared (Figure 6) by Duschinsky and co-workers.[85,125,133,134,218,245,246] The structure-activity relationships[151] of the interaction of FUDR and its derivatives with the key enzymes in its mode of action were tentatively given and the important points are illustrated in Figure 7.

In addition to the biochemical aspects of 5-fluorouracil, the molecule undergoes reactions typical of uracil. It was concluded from spectral investigations[247] that the monoanionic form of 5-fluorouracil consists of an equilibrium mixture of two tautomeric forms I and II as shown in Scheme 6. In aqueous solution, form I, corresponding to dissociation the proton on the more electronegative ring nitrogen,

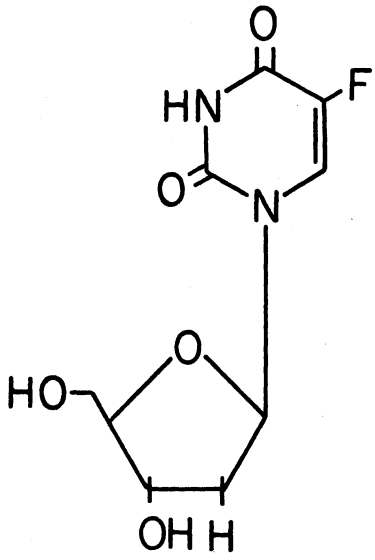

H2NCH2CHFCO2H
+
H2NCNH2
+
CO2
H2NCNHCH2CHFCO2H
+
H2NCNHCH2CHFCO2H
HN
F
O
N
H
FUR
FURP
FUDRP
F-RNA
FURPPP
UDRP
TDRP
TTP
DNA

Scheme 5



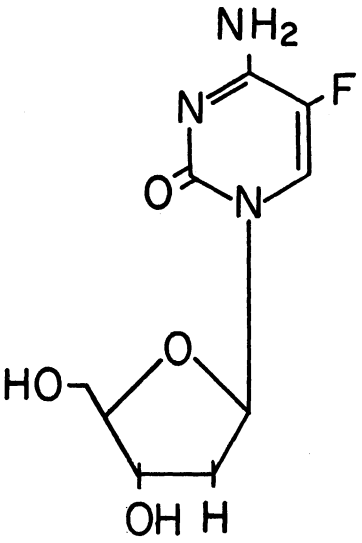
FUR

49



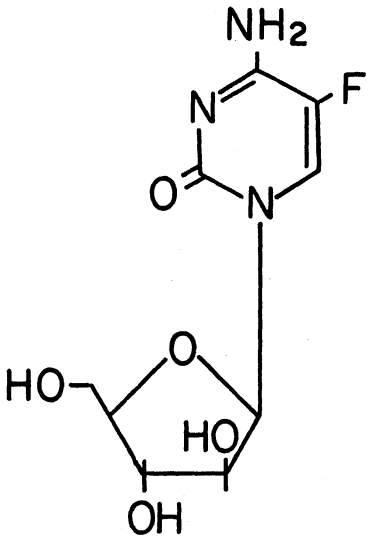
FUDR

50



FCDR

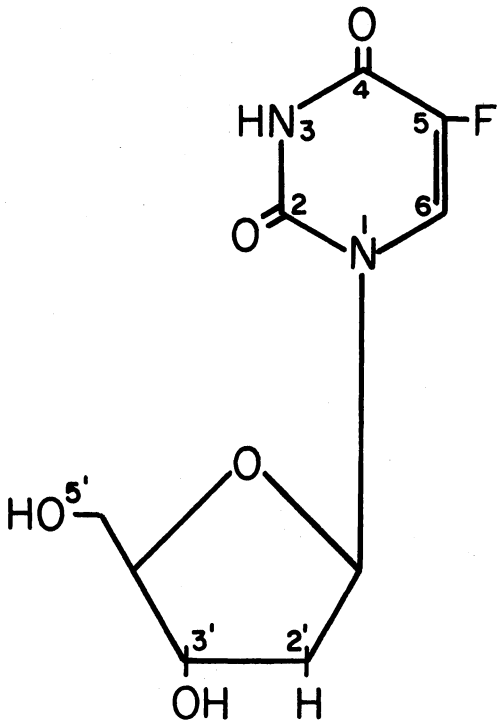
51



ara-FCDR

52

Figure 6. The Structures of Fluorinated Pyrimidine Nucleosides



N-1. Necessity unknown: to be tested

O-2. Necessary for activity

N-3. Must be unsubstituted and with proton of correct pK_a

O-4. Probably necessary. Cannot be methylated

C-5. Size of substituent determines incorporation into DNA or RNA and electron properties

C-6. Must be unsubstituted. 6-AzaFU could not be synthesized. 6-$AZAF_3$TDR inactive: too acidic

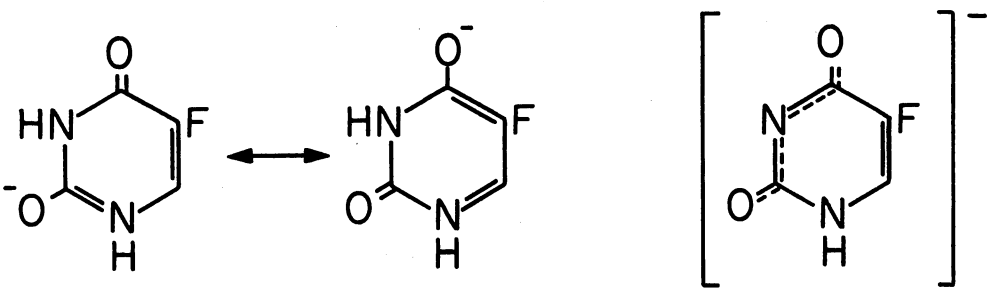
H-2' H needed for DNA action, OH for RNA. 2'-Ara-hydroxyl gives 4-amino compounds desirable properties

H-3' Can be blocked with methoxyl group and retains slight TdR-kinase and TMP-synthetase activity, but prevents nucleoside phosphorylase cleavage

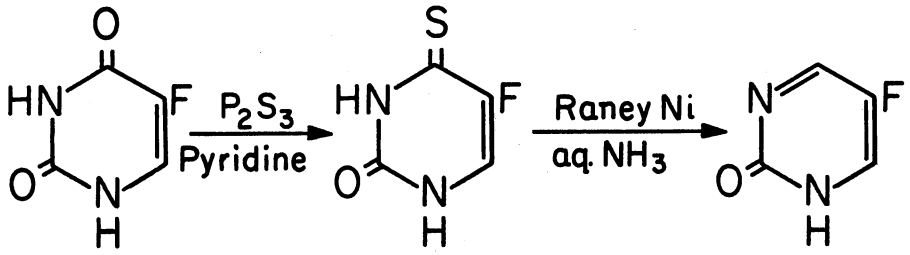
H-5' Must be unsubstituted. However, some 5'-halo nucleosides inhibit TMP-kinase

Furanose-O. Probably necessary, but not adequately tested.

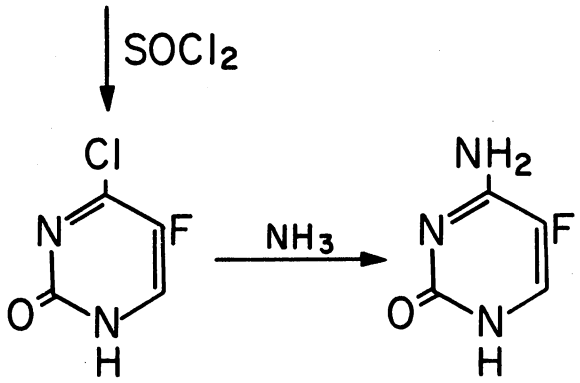
Figure 7. Structure-Activity Relationships of the Interaction of 5-Fluoro-2'-deoxyuridine and Its Derivatives with the Key Enzymes Involved in Its Mode of Action



I II



42 53 54

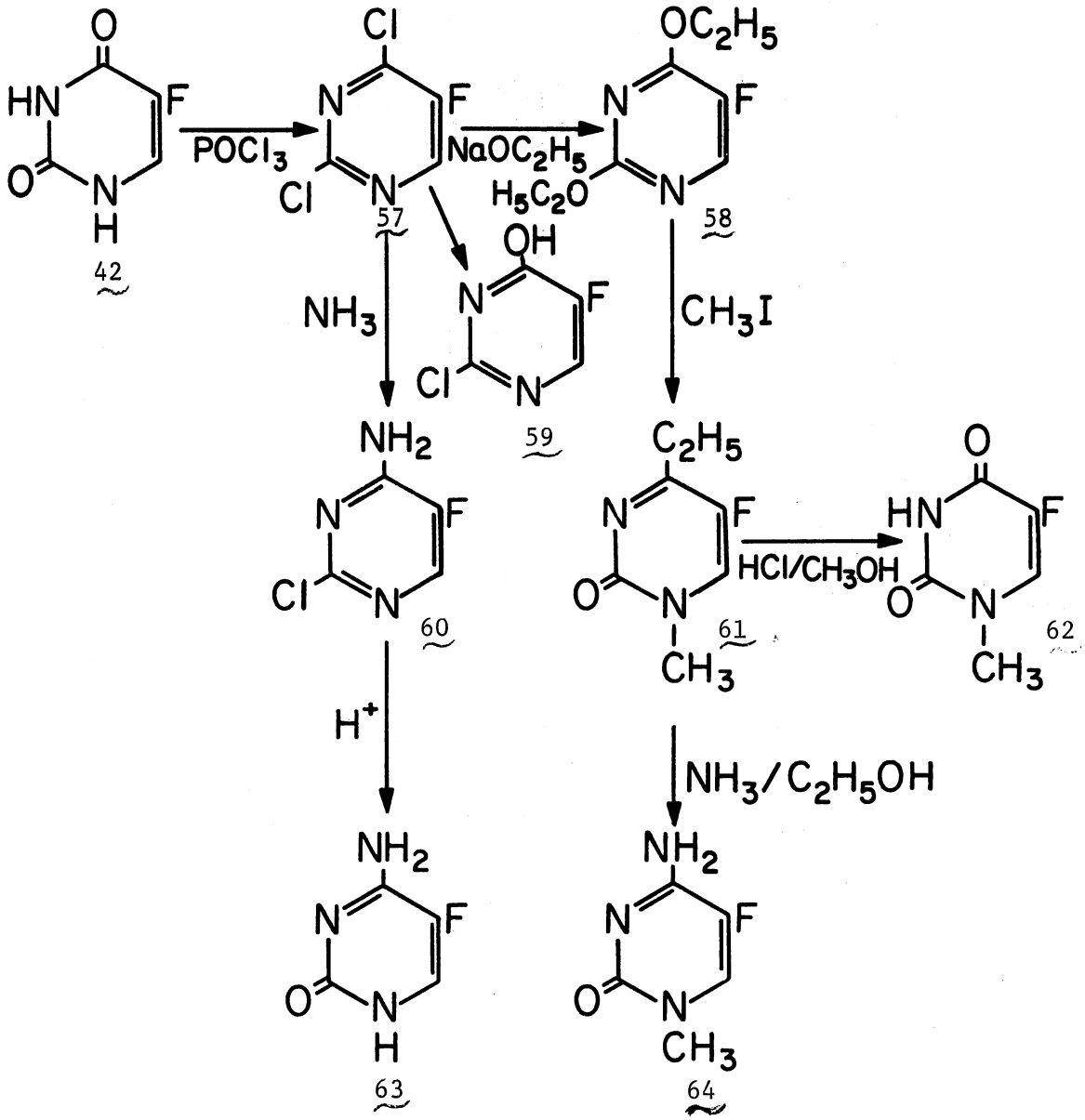


55 56

Scheme 6

comprises 63% of the mixture, but form II is predominant in solvents of lower dielectric constant.

The presence of the fluorine at C(5) increases the susceptibility of the 4-position to nucleophilic attack as compared to attack at C(2). Preferential reactions at the 4-position are partially illustrated in Scheme 6 and 7.[20,87,88,242] The synthesis and testing of several derivatives of 5-FU were the result of anticancer properties possessed by 5-FU.[124]

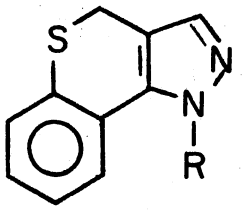

POCl3
NaOC2H5
NH3
CH3I
H+
HCl/CH3OH
NH3/C2H5OH
42
57
58
59
60
61
62
63
64

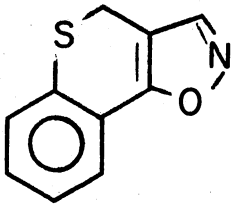
Scheme 7

CHAPTER II

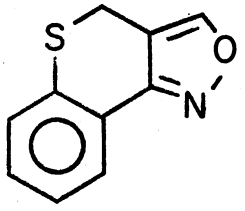
RESULTS AND DISCUSSION

The results of this work are divided into three parts. Synthesis of pyrazoles 65, 66, 67, 81, and 85, isoxazoles 68, 84, 87, and 88, pyrazol-3-ones 79 and 83, and sulfones 70, 71, 72, and 73 constitutes

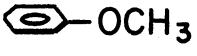


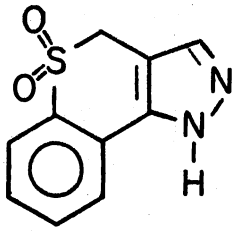


68

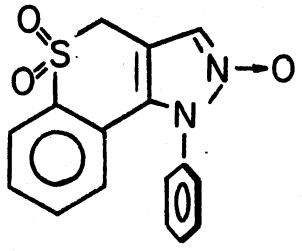


69

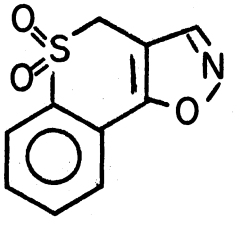
65 R = H ; 66 R =  ; 67 R = 



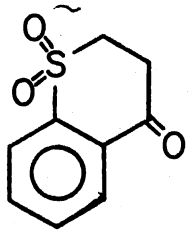
70



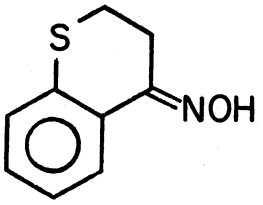
71



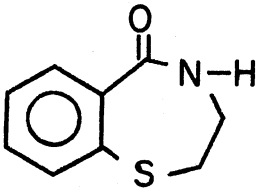
72



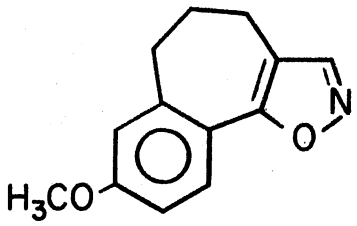
73



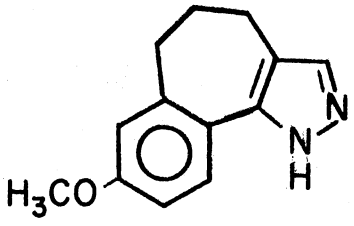
74



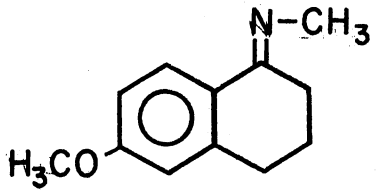
75



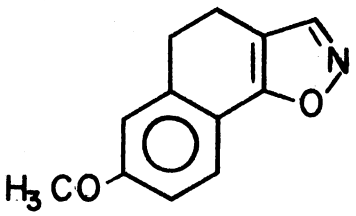
84



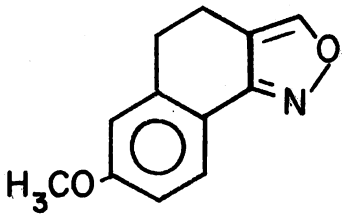
85



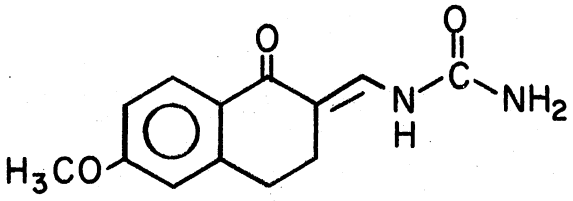
86



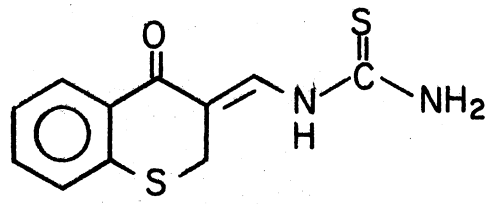
87



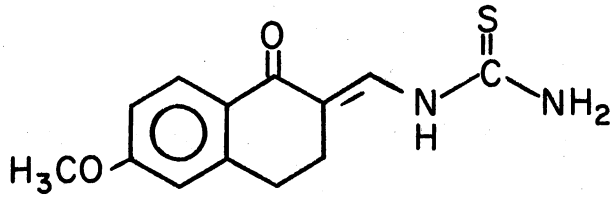
88



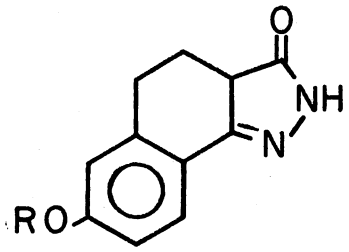
76



78

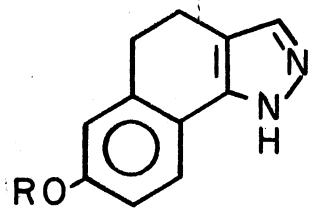


77



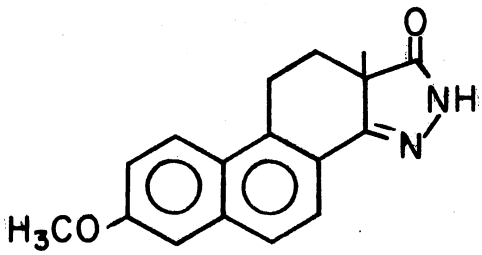
79 R = CH_3

80 R = H



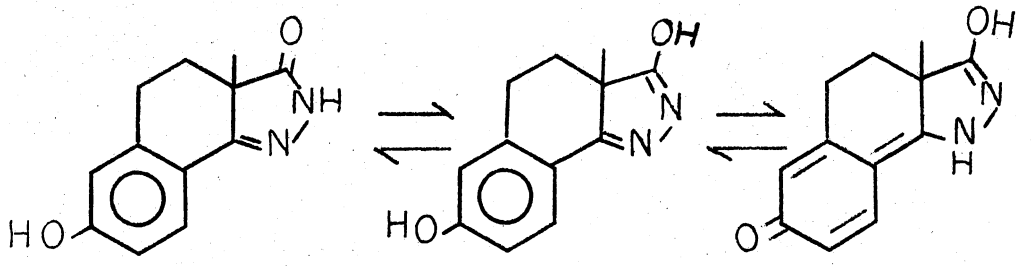
81 R = CH_3

82 R = H

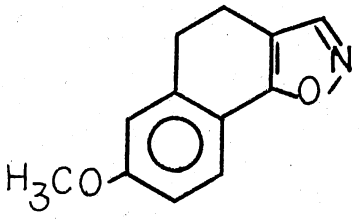


83

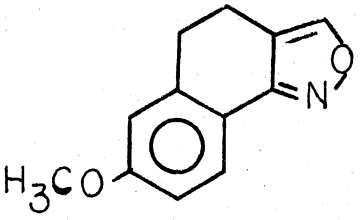
the first part. A second study was concerned with a rather comprehensive PMR and UV spectral analysis of hydroxy pyrazol-3-one 80. This was considered necessary in order to determine whether tautomeric forms might exist under several conditions of pH. A ^{13}CMR study was also initiated to distinguish the isomeric nature of structures 87 and 88.



80



87



88

The PMR study has been published in part.[239]

The third and final area of concentration concerned the study of complexation between selected heterocycles and certain acceptor candidates, notably 5-fluorouracil, 1,3,5-trinitrobenzene, 4-flourophenylacetonitrile, and 3,4-dihydroxybenzoic acid. Both ultraviolet and NMR spectral analyses were performed to examine the structure of the complex in solution.

A careful NMR (Table XI) and mass spectral analysis supported the proposed structure of each of the new compounds. Rather than obtain

TABLE X

PERCENTAGE YIELDS, AND MELTING POINTS OF STARTING MATERIALS, INTERMEDIATES, AND PRODUCTS

Compound Name	Cpd.	m.p., °C	Yield, %
Thiochroman-4-one	90	28-30	
2(Hydroxymethylene)thiochroman-4-one	94		87.5
4H[1]Benzothiopyrano[3,4-d]isoxazole	68	71-73	84.3
1,4-Dihydro[1]benzothiopyrano[4,3-c]-pyrazole	65	168.5-170	93.8
1,4-Dihydro-1-phenyl[1]benzothiopyrano-[4,3-c]pyrazole	66	169-171	88.7
1,4-Dihydro-1-(p-methoxyphenyl)-[1]-benzothiopyrano[4,3-c]pyrazole	67	145-146	29.0

TABLE X (Continued)

Compound Name	Cpd.	m.p., °C	Yield, %
4H-[1]benzothiopyrano[3,4-d]isoxazole-5,5-dioxide	72	170-172	94.8
1,4-Dihydro[1]benzothiopyrano[4,3-c]-pyrazole-5,5-dioxide	70	249-250	64.4
1,4-Dihydro-1-phenyl[1]benzothio-pyrano[4,3-c]pyrazole-2,5,5-trioxide	71	211-212	99.8
Thiochroman-4-one-1,1-dioxide	73	131-133	65.7
1-[4,Oxothiochroman-3-yl)-methylene]-2-thiourea	78	184-186	31.4
6-Methoxy-1-tetralone	89		
2-Hydroxymethylene-6-methoxy-1-tetralone	93	66-68	95.7

TABLE X (Continued)

Compound Name	Cpd.	m.p., °C	Yield, %
[(1,2,3,4-Tetrahydro-6-methoxy-1-oxo-2-naphthyl)methylene]urea	76	235-237	83.2
1-[1,2,3,4-Tetrahydro-6-methoxy-1-oxo-2-naphthyl)methylene]-2-thiourea	77	225-227	99.3
6-Methoxy-2-methyl-2-carbomethoxy-1-oxo-1,2,3,4-tetrahydronaphthalene	97	91-92.5	82.0
2,3a,4,5-Tetrahydro-3a-methyl-7-methoxy-3H-benz[g]indazole-3-one	79	218-219	90.5
2,10,11,11a-Tetrahydro-7-methoxy-11a-methyl-1H-phenanthro[1,2-c]-pyrazol-1-one	83	258-260	70.6
4,5-Dihydro-7-methoxynaphth[2,1-d]-isoxazole	87	59-61	88.0

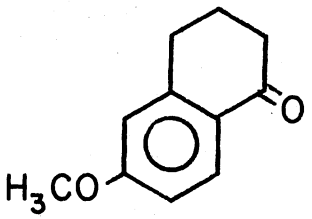
TABLE X (Continued)

Compound Name	Cpd.	m.p., °C	Yield, %
Thiochroman-4-one oxime	74	98-100	91.7
2(Hydroxymethylene)2-methoxybenzo-suberone	96		95.9
1,4,5,6-Tetrahydro-8-methoxybenzo-[6,7]cyclohepta[1,2-c]pyrazole	85	101-103	62.9
5,6-Dihydro-8-methoxy-4H-benzo-[3,4]cyclohepta[1,2-d]isoxazole	84	52-53	81.6
2(Hydroxymethylene)6-methoxy-1-indanone	95	149-150	94.0
2(Hydroxymethylene)5,6-dimethoxy-1-indanone	98	151	96.1
N-methyl-6-methoxy-1-tetralone imine	86	53-55	97.9

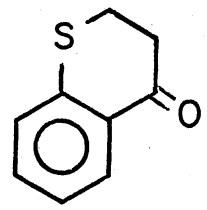
elemental analyses on each compound, the molecular ion (M^+) was peak matched via mass spectral analysis. Percentage yields and melting points of starting materials, isolated intermediates, and products are found in Table X. The significance of these compounds as chemotherapeutic agents is of special interest in view of the biological activities found in compounds possessing similar functionalities (refer to Chapter I).

Synthetic Results

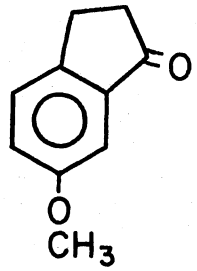
In the course of this investigation, several key starting materials were required, namely the 1-tetralone (89), thiochromanone (90), the indanone 91, and the benzosuberone 92. Treatment of ketone 89, 90, 91, or 92 with ethyl formate in the presence of sodium methoxide resulted in



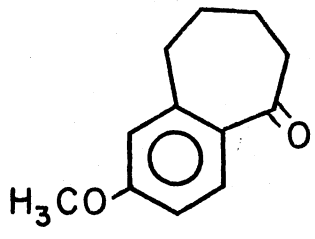
89



90



91



92

TABLE XI

NMR ANALYSIS OF STARTING MATERIALS, INTERMEDIATES, AND PRODUCTS

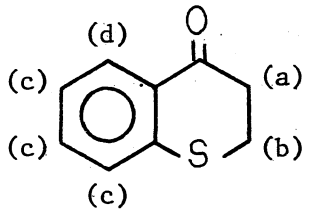
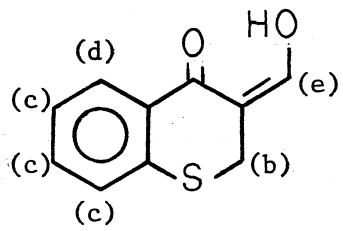
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	90		$DCCl_3$	2.86 (t)	2	CH_2 (a)
				3.17 (t)	2	CH_2 (b)
				7.00-7134 (m)	3	ArH (c)
				8.06 (d)	1	ArH (d)
	94		$DCCl_3$	3.59 (s)	2	CH_2 (b)
				7.04-7.34 (m)	3	ArH (c)
				7.94 (d)	1	ArH (c)
				8.30 (s)	1	C=CH (e)

TABLE XI (Continued)

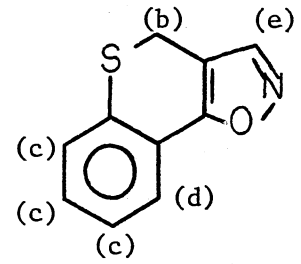
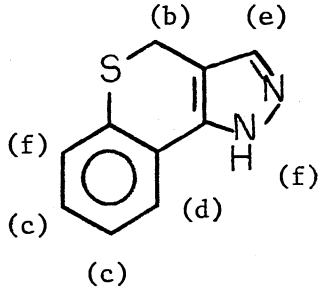
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	68		$DCCl_3$	4.00 (s)	2	CH_2 (b)
				7.10-7.40 (m)	3	ArH (c)
				7.60-7.80 (m)	1	ArH (d)
				8.12 (s)	1	N=C<u>H</u> (e)
	65		Acetone-d_6	2.70 (bs)	1	NH (f)
				3.99 (s)	2	CH_2 (b)
				7.04-7.30 (m)	3	ArH (c)
				7.48 (s)	1	N=C<u>H</u> (e)
				7.79-7.80 (m)	1	ArH (d)

TABLE XI (Continued)

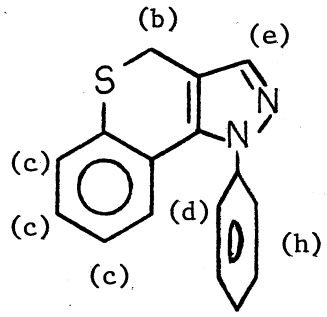
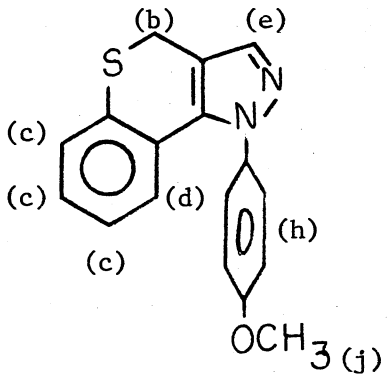
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	66		$DCCl_3$	3.90 (s)	2	CH_2 (b)
				6.82-7.17 (m)	3	ArH (c)
				7.41 (s)	6	ArH (d) and (h)
				7.56 (s)	1	N=CH (e)
	67		$DCCl_3$	3.83 (s)	3	OCH_3 (j)
				3.88 (s)	2	CH_2 (b)
				6.82-7.50 (m)	9	ArH (h), (c), (d) and (e)

TABLE XI (Continued)

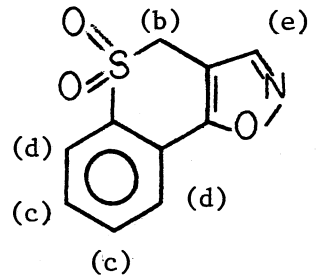
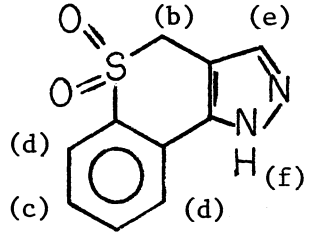
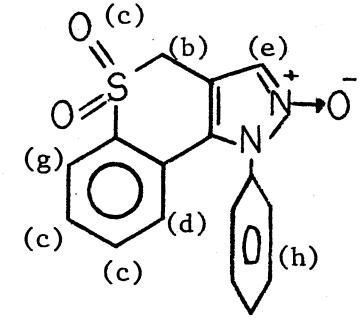
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	72		$DCCl_3$	4.43 (s)	2	CH_2 (b)
				7.63-7.80 (m)	2	ArH (c)
				7.80-8.10 (m)	2	ArH (d)
				8.32 (s)	1	N=C<u>H</u> (e)
	70		DMSO-d_6	3.26 (bs)	1	NH (f)
				4.68 (s)	2	CH_2 (b)
				7.46-8.40 (m)	5	ArH (c), (d) and N=C<u>H</u> (e)
	71		$DCCl_3$	4.41 (s)	2	CH_2 (b)
				6.84-6.94 (m)	1	ArH (d)
				7.25-7.47 (m)	7	ArH (c) and (h)
				7.70 (s)	1	N=C<u>H</u> (e)

TABLE XI (Continued)

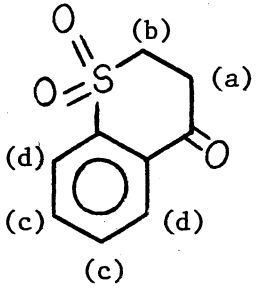
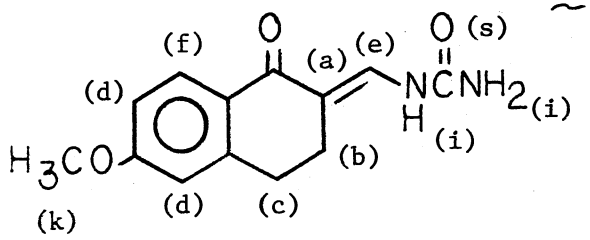
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	71			8.01-8.12 (m)	1	ArH (g)
	73		$DCCl_3$	3.36 (t)	2	CH_2 (a)
				3.66 (t)	2	CH_2 (b)
				7.60-7.90 (m)	2	ArH (c)
				7.92-8.17 (m)	2	ArH (d)
	76		DMSO-d_6	2.57-2.70 (m)	2	CH_2 (b)
				2.84 (t)	2	CH_2 (c)
				3.36 (s)	2	NH_2 (i)
				3.83 (s)	3	CH_3 (k)
				6.82-7.00 (m)	2.5	ArH (d) and $-\overset{O}{\overset{\|}{C}}-CH$ (a)
				7.80-7.90	1	ArH (f)

TABLE XI (Continued)

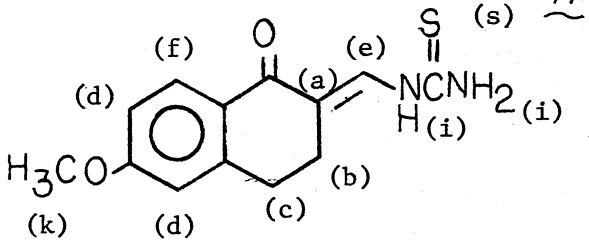
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	76			[Absorption at 6.38 (s), 7.44 (s), 8.10 (s), 9.90 (d), and 10.84 (d) may be small contributions from different tautomeric forms; all together they correspond to 1.5 protons and thus total number of protons was found to be 14, which was the expected value.]		
	77		DMSO-d_6	2.60-2.80 (m)	2	CH_2 (b)
				2.80-3.02 (bd)	2	CH_2 (c)
				3.32 (s)	2	NH_2 (i)
				3.84 (s)	3	CH_3 (k)
				6.84-7.00 (m)	2.5	ArH (d) and $-\overset{O}{\overset{\|}{C}}-C\underline{H}$ (a)
				7.84 (bd)	1	ArH (d)

TABLE XI (Continued)

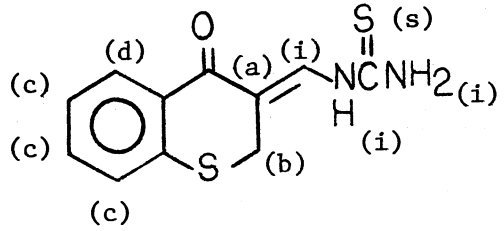
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	77			7.93 (s)	1	N=CH (e)
				8.67 (bs)	0.5	SH (j)
				8.43 (d), 9.83 (d), and 11.78 (d) may be small contributions from different tautomeric structures.		
	78		DMSO-d_6	2.80-3.80 (vb)	1	NH (i)
				3.84-4.09 (s and d)	2	CH_2 (b)
				7.20-7.57 (m)	3	ArH (c)
				7.92-8.50 (m)	2.5	ArH (d), N=CH (e) and -C(=O)-CH (a)
				8.90 (bs)	0.8	SH (j)
				[10.20-10.32 (bd) and 11.60-11.74 (bd) may be small contributions (0.8 proton) from:		

TABLE XI (Continued)

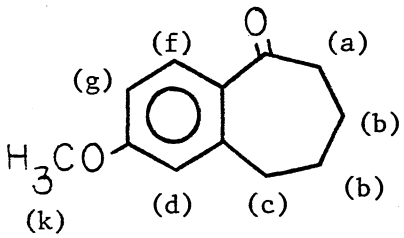
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	78			S N—C H(i) SH (f) N=C ; H_2N—C S NH=C SH (f) and		etc.]
 	92		$DCCl_3$	1.70-1.97 (m)	4	CH_2-CH_2 (b)
				2.70 (t)	2	CH_2 (c)
				2.90 (t)	2	CH_2 (a)
				3.83 (s)	3	O—CH_3 (k)
				6.70 (d)	1	ArH (d)
				6.80 (dd)	1	ArH (g)
				7.78 (d)	1	ArH (f)

TABLE XI (Continued)

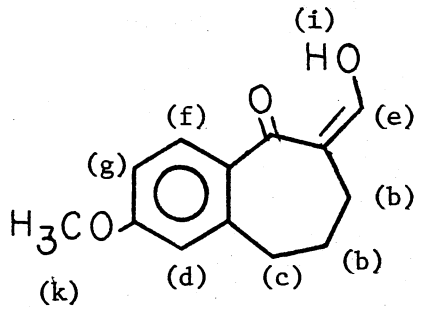
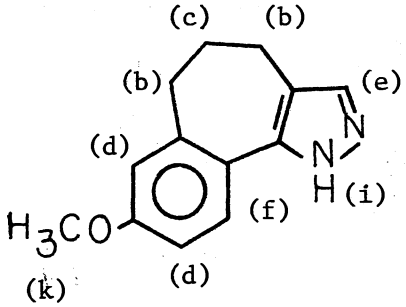
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	96		$DCCl_3$	1.76-2.20 (m)	4	CH_2—CH_2 (b)
				2.62 (m)	2	CH_2 (c)
				3.78 (s)	3	O—CH_3 (k)
				6.70 (d)	1	ArH (d)
				6.80 (dd)	1	ArH (g)
				7.56 (d)	1	ArH (f)
				7.94 (s)	1	C=C<u>H</u> (e)
	85		$DCCl_3$	1.77-2.18 (m)	2	CH_2 (c)
				2.64-2.92 (m)	4	CH_2—C—CH_2 (b)
				3.78 (s)	3	O—CH_3 (k)
				6.62-6.84 (m)	2	ArH (d)
				7.41 (s)	1	N=C<u>H</u> (e)
				7.64 (d)	1	ArH (f)
				10.50-11.02 (bs)	1	NH (i)

TABLE XI (Continued)

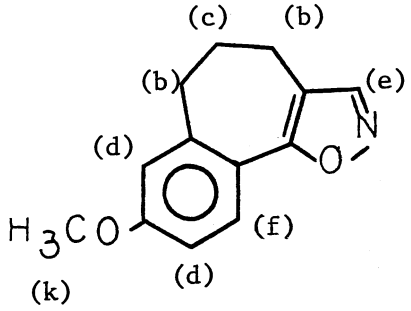
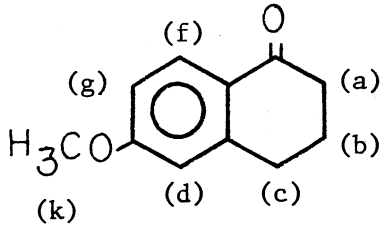
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	84		$DCCl_3$	1.96 (q)	2	CH_2 (c)
				2.42-3.00 (m)	4	CH_2—C—CH_2 (b)
				3.77 (s)	3	O-CH_3
				6.56-6.92 (m)	2	ArH (d)
				7.83 (d)	1	ArH (f)
				8.06 (s)	1	N=C<u>H</u> (e)
	89		$DCCl_3$	2.10 (q)	2	CH_2 (b)
				2.60 (t)	2	CH_2 (c)
				2.91 (t)	2	CH_2 (a)
				3.83 (s)	3	OCH_3 (k)
				6.69 (d)	1	ArH (d)
				6.80 (dd)	1	ArH (g)
				7.98 (d)	1	ArH (f)

TABLE XI (Continued)

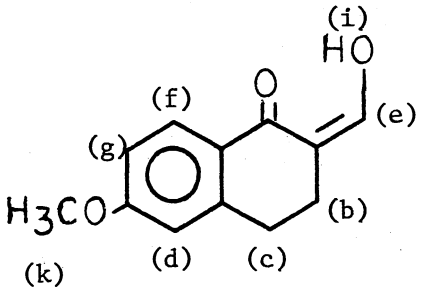
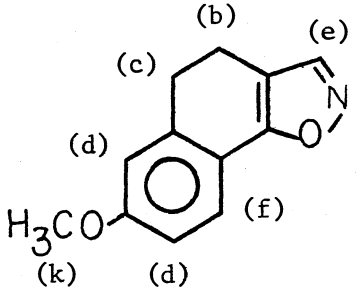
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	93		$DCCl_3$	2.54 (t)	2	CH_2 (c)
				2.87 (t)	2	CH_2 (b)
				3.84 (s)	3	OCH_3 (k)
				6.72 (d)	1	ArH (d)
				6.84 (dd)	1	ArH (g)
				7.91-7.98 (m)	1	ArH (f)
				14.40-14.76 (bs)	1	C=COH (i)
	87		$DCCl_3$	2.63-2.82 (t)	2	CH_2 (c)
				2.84-3.10 (t)	2	CH_2 (b)
				3.83 (s)	3	OCH_3 (k)
				6.77-6.90 (m)	2	ArH (d)
				7.56-7.67 (m)	1	ArH (f)
				8.11 (s)	1	N=C<u>H</u> (e)

TABLE XI (Continued)

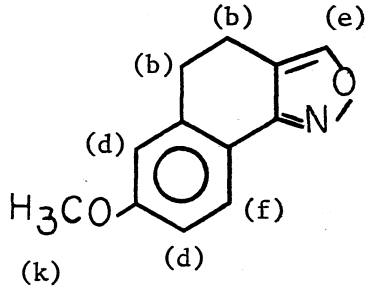
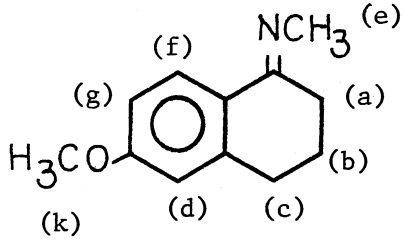
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	88		$DCCl_3$	2.54-2.98 (m)	4	CH_2-CH_2 (b)
				3.75 (s)	3	OCH_3 (k)
				6.51-6.90 (m)	2	ArH (d)
				7.84 (d)	1	ArH (f)
				8.05 (s)	1	N=C<u>H</u> (e)
	86		$DCCl_3$	1.92 (q)	2	CH_2 (b)
				2.53 (t)	2	CH_2 (c)
				2.77 (t)	2	CH_2 (a)
				3.27 (s)	3	N-CH_3 (1)
				3.79 (s)	3	OCH_3 (k)
				6.61 (d)	1	ArH (d)
				6.78 (dd)	1	ArH (g)
				8.04 (d)	1	ArH (f)

TABLE XI (Continued)

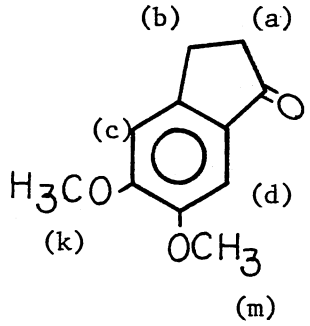
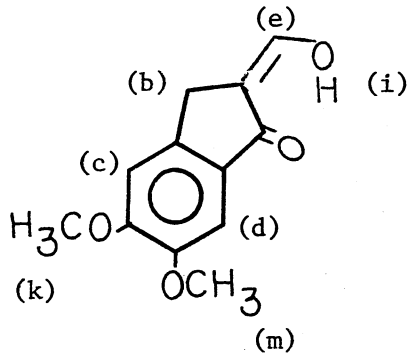
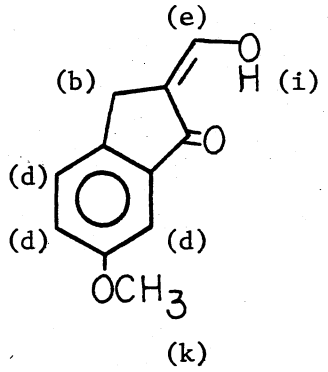
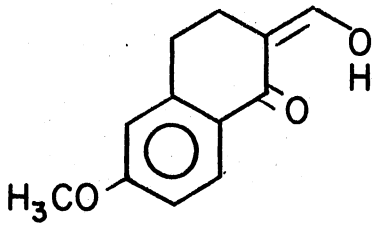
Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	134		$DCCl_3$	2.64 (t)	2	CH_2 (b)
				3.01 (t)	2	CH_2 (a)
				3.89 (s)	3	OCH_3 (k) or (m)
				3.93 (s)	3	OCH_3 (k) or (m)
				6.87 (s)	1	ArH (c)
				7.16 (s)	1	ArH (d)
	98		DMSO-d_6	3.51 (s)	2	CH_2 (b)
				3.84 (s)	3	OCH_3 (k) or (m)
				3.89 (s)	3	OCH_3 (k) or (m)
				5.51 (bs)	1	OH (i)
				7.10 (s)	1	ArH (c)
				7.16 (s)	1	ArH (d)

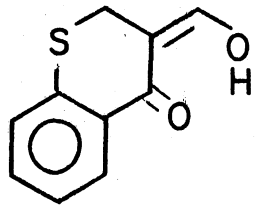
TABLE XI (Continued)

Structure	Cpd.	Plate	Solvent	δ (p.p.m.)[a]	Integr.	Assignments
	98			7.67 (s)	1	C=CH (e)
	95		$DCCl_3$	3.52 (s)	2	CH_2 (b)
				3.85 (s)	3	OCH_3 (k)
				7.10-7.40 (m)	3	ArH (d)
				7.63 (s)	1	C=CH (e)
				8.74 (bs)	1	OH (i)

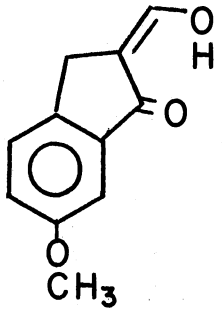
the formation of the corresponding hydroxymethylene compounds.[1,142] 93, 94, 95, or 96. Though the existence of tautomeric forms (keto-enol)[238]



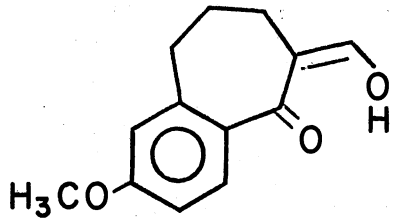
93



94



95



96

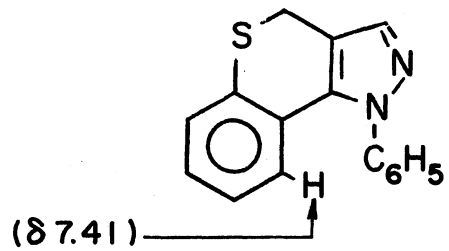
in this type of compound was well recognized, the keto-hydroxymethylene form predominates in all cases of our study. This predominate existence of the enol form was inferred by the absence of aldehydic proton signal in the NMR spectrum and the presence of a definite hydroxyl group absorption in the IR spectrum (Plates XVIIIb and XXVb). This is surprising in view of published work on simple systems[238] (Table XI). For example, our data contradict the results of Terinski and Kozluk[238] who reported that the amount of keto-hydroxymethylene form decreased with ring size in the order of $C_5 > C_7 > C_8 > C_6 > C_9$.

Hydroxymethylene ketones of this general type are known to cyclize to hydroindazoles by treatment with hydrazine.[1,143,236] Accordingly,

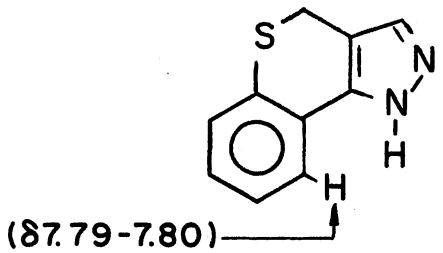
the compounds 93, 96, and 94 were treated with 95% hydrazine to give the corresponding pyrazoles in very good yields (Schemes 8, 9 and 10). Since two tautomeric forms were conceivable,[40,146,168] a careful PMR spectral analysis was performed; it gave a pattern consistent with the existence of the 1H form rather than the 2H form (Table XI) as shown in Schemes 8, 9 and 10.

Substituted hydrazines were treated with the appropriate hydroxymethylene compound to give a specific product, the N(1) derivative,[64] which was also consistent with our earlier results. The syntheses of the phenylpyrazole 66 and the p-methoxyphenylpyrazole 67 were achieved by treating ketone 94 with phenylhydrazine and p-methoxyphenylhydrazine respectively, in glacial acetic acid medium (Scheme 10). The identities of the products were verified by elemental analyses, and spectral analyses (Tables X and XI).

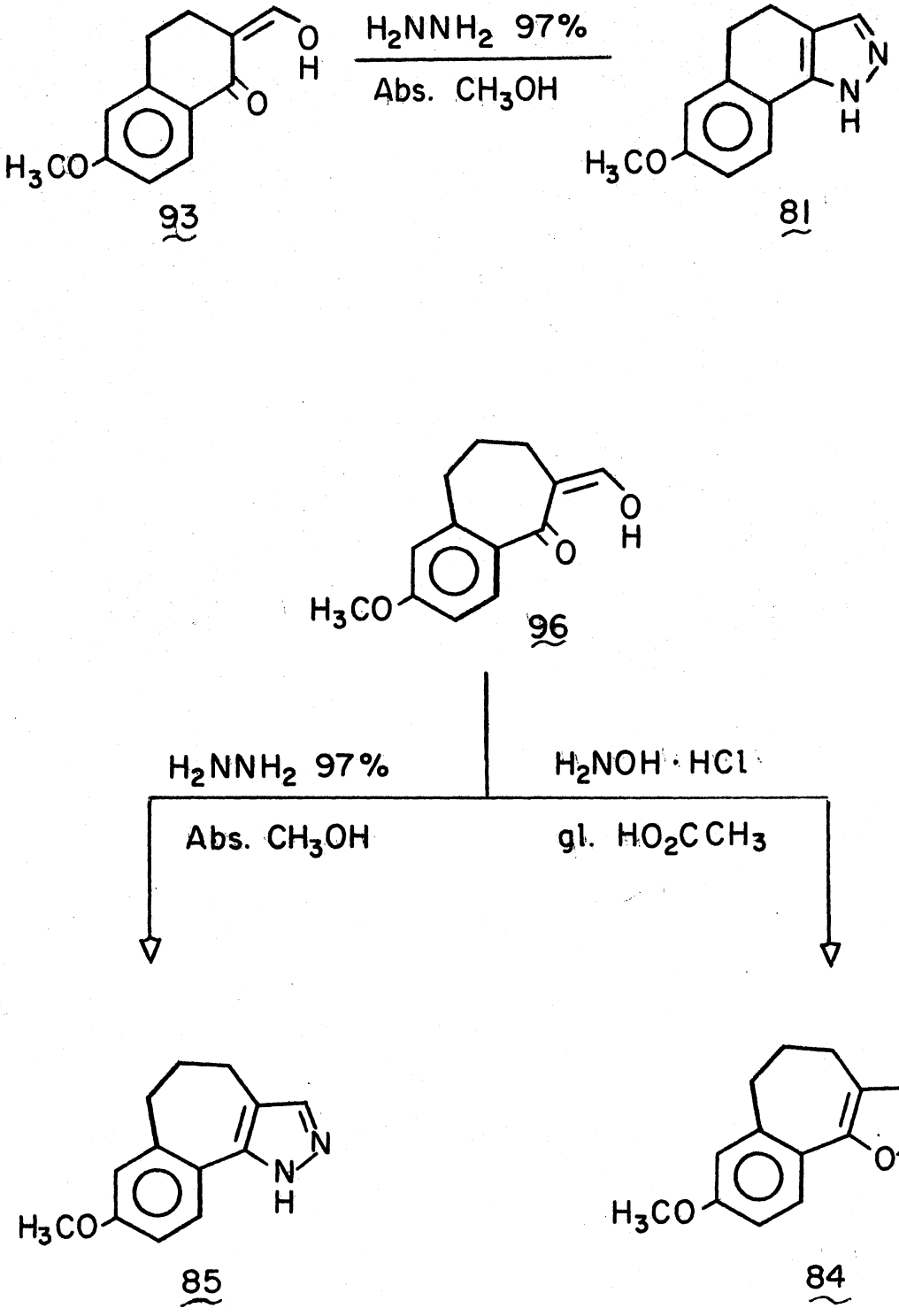
In pyrazole formation the imtermediate is presumably a hydrazone derivative which might lead to the formation of N(1) or N(2) substituted pyrazoles, perhaps depending upon the difference in the nucleophilic character of the two nitrogen atoms in the substituted hydrazines.[17,18,64] The location of phenyl group at N(1) is further supported by the upfield shifts of the peri hydrogen, marked H(9) (Table XI).



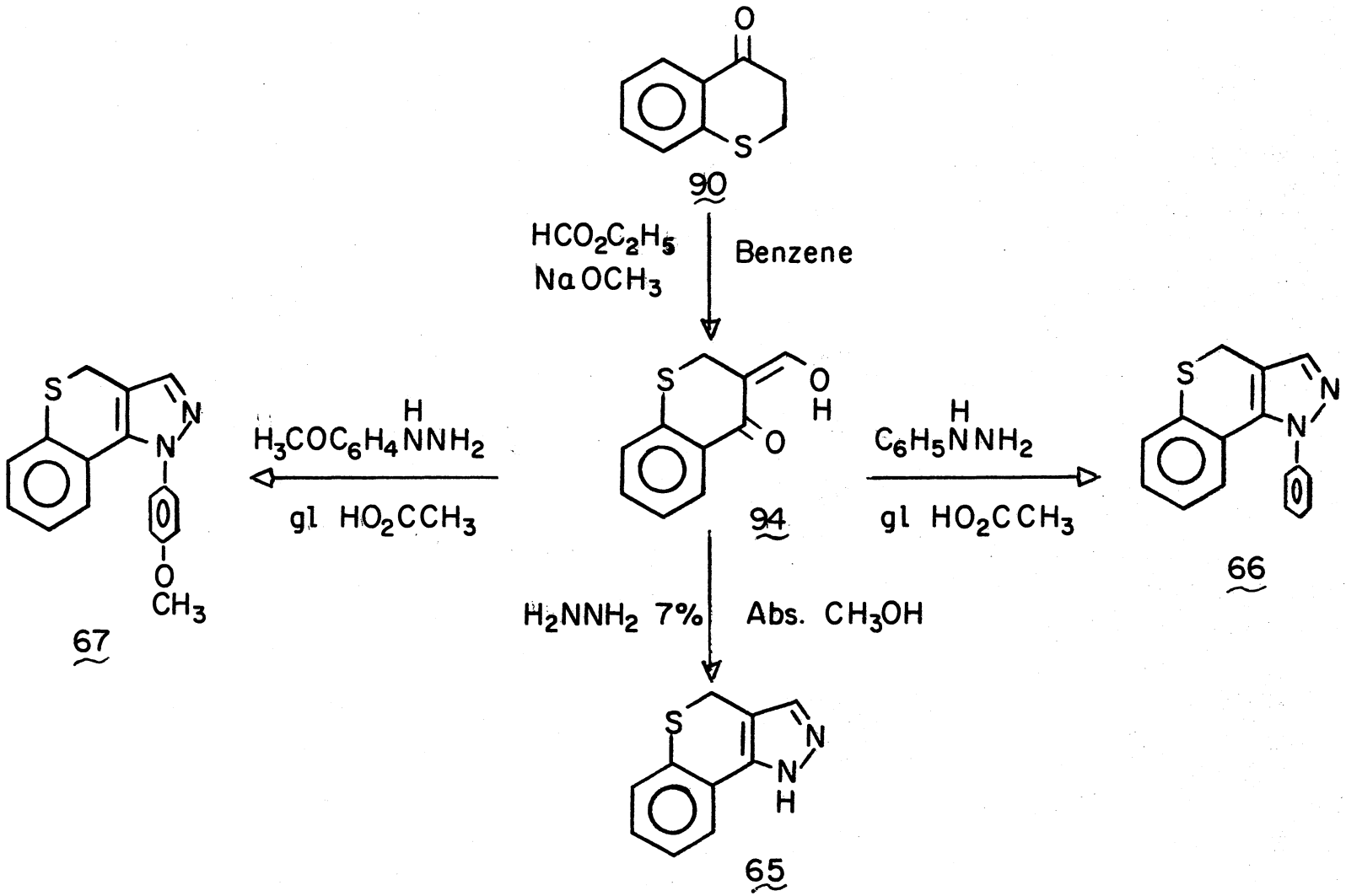
66



65

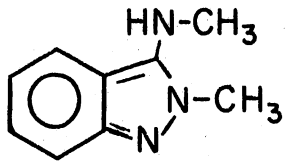

H2NNH2 97%
Abs. CH3OH
H3CO
O
H
N
N
H
93
81
96
H2NNH2 97%
Abs. CH3OH
H2NOH · HCl
gl. HO2CCH3
85
84

Scheme 9

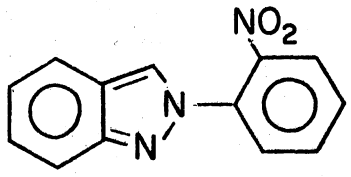

O
S
90
$HCO_2C_2H_5$
NaOCH$_3$
Benzene
S
O
H
O
94
$H_3COC_6H_4\overset{H}{N}NH_2$
gl HO_2CCH_3
$C_6H_5\overset{H}{N}NH_2$
gl HO_2CCH_3
S
N
N
O
CH_3
67
S
N
N
O
66
H_2NNH_2 7%
Abs. CH_3OH
S
N
N
H
65

Scheme 10

Synthesis of N-substituted pyrazoles was actually stimulated by the observation that certain tetrahydroindazoles such as 99[231] and 100[203] exhibit analgesic and herbicide activities, respectively.



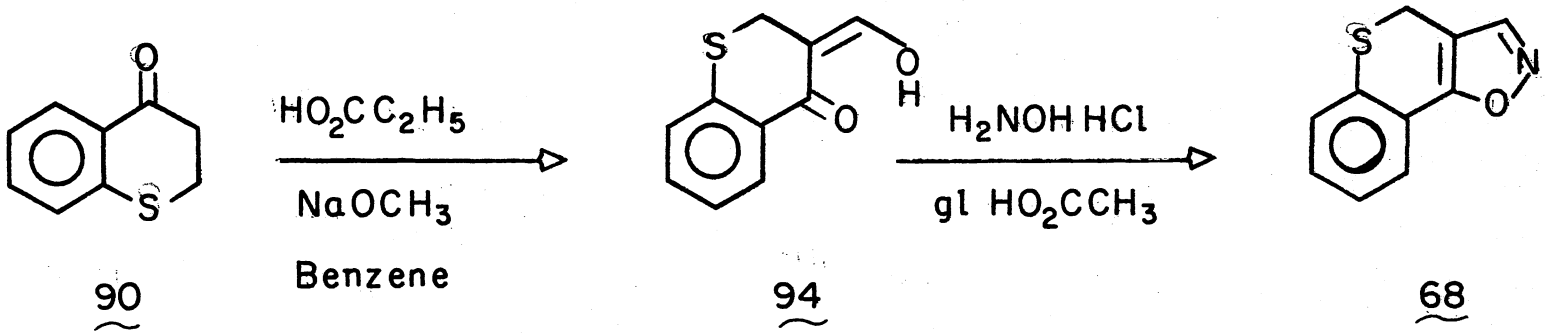
99



100

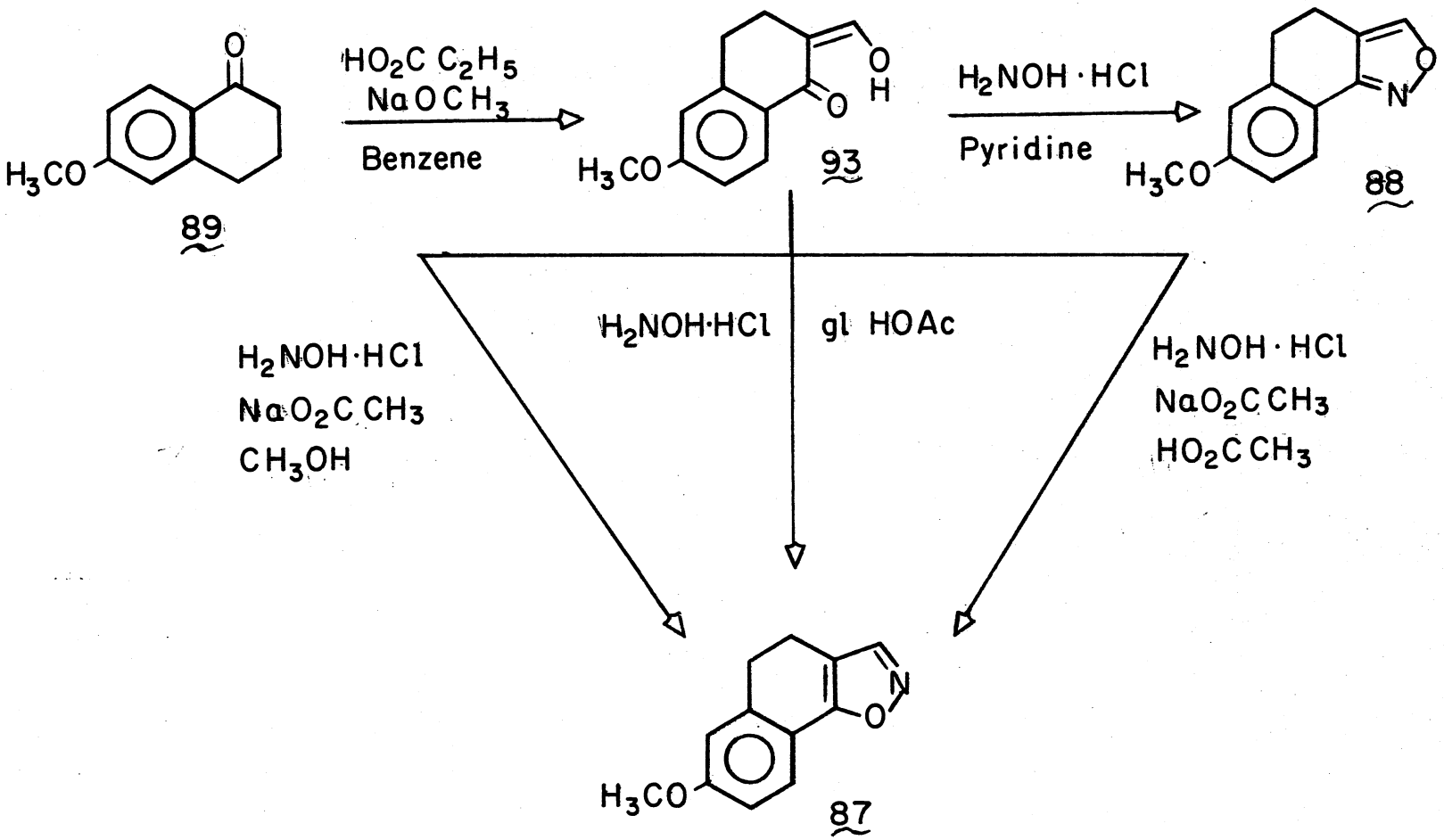
The synthesis of isoxazole derivatives 68, 84, and 87 was accomplished via treatment of the required hydroxymethylene compounds with hydroxylamine hydrochloride in glacial acetic acid (Schemes 9, 11, and 12).[111] If the reaction was performed in pyridine, isomeric [1,2-c]-isoxazole 88 was obtained (Scheme 12). ^{13}CMR spectral analysis strongly supports the structures given for the two isomeric isoxazoles. The ^{13}CMR data and possible mechanism of formation of the two isomers is discussed later in this chapter (page 101).

During the investigation of the reaction of certain α-hydroxymethylene ketones with hydroxylamine hydrochloride, a remarkable difference in behavior was noticed. In cyclic systems where n = 1 (cyclopentanone derivatives, 95 and 98), the hydroxylamine nitrogen became a bridge between two ketonic rings, while the compounds (93, 94, and 96) where n = 2 and/or 3 were smoothly transformed into the corresponding isoxazoles. This general phenomenon was first observed by Johnson and Shelberg[141] in simple systems. The resistance to formation of intermolecular condensation products in the case of cyclopentanone derivatives may be due to strain in the 5-5-6 fused ring system. It was

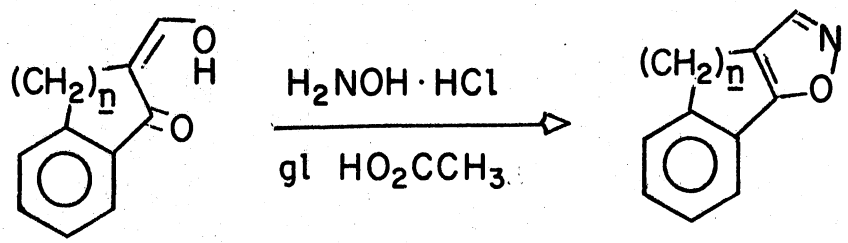


90 94 68

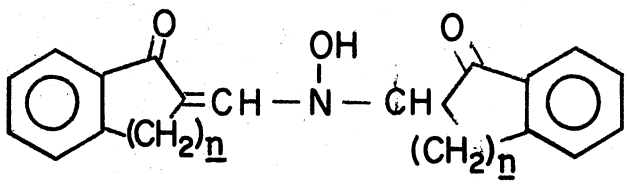
Scheme 11


O
H3CO
89
HO2C C2H5
NaOCH3
Benzene
O
H
O
H3CO
93
H2NOH·HCl
Pyridine
O
N
H3CO
88
H2NOH·HCl
NaO2C CH3
CH3OH
H2NOH·HCl
gl HOAc
H2NOH·HCl
NaO2C CH3
HO2C CH3
N
O
H3CO
87

Scheme 12

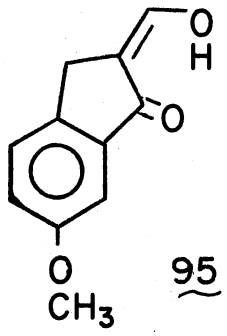


where n = 2 or 3

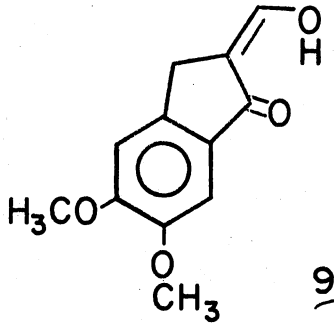


where n = 1

also noted that 95 and 98 failed to condense with hydrazine to give the corresponding pyrazole. This anomalous behavior is probably also



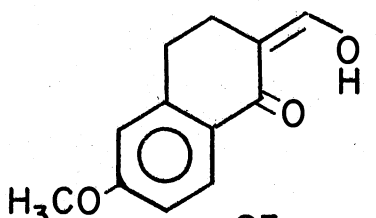
95



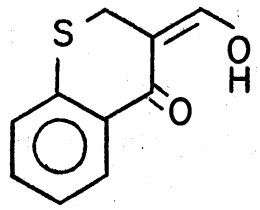
98

attributed to strain effects. Analogous to the formation of isoxazoles, formation of pyrazole ring from the six and seven membered ring compounds (93, 94, and 96) was done with greater ease and efficiency (Table X).

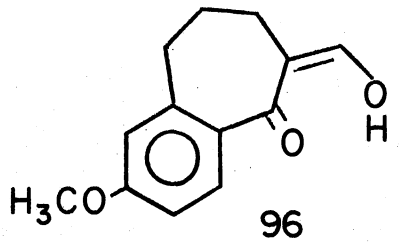
Formation of the sulfone derivatives 70, 71, 72, and 73 was successfully achieved by the reaction at room temperature between the corresponding compounds 65, 66, 68 and 90, respectively, and 30% hydrogen peroxide in glacial acetic acid (Scheme 13). In the case of



93



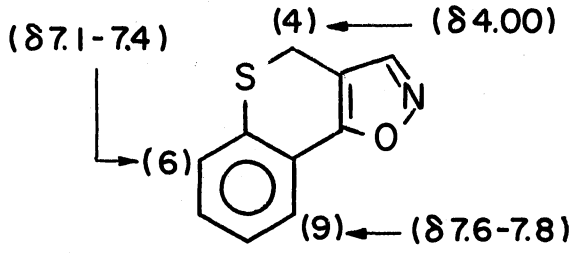
94



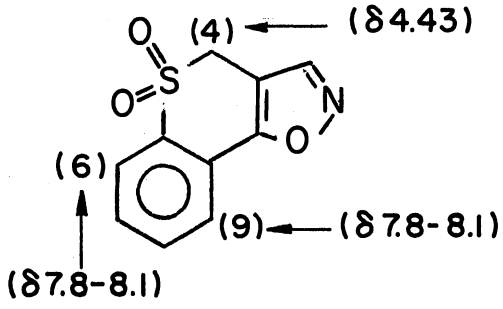
96

the phenyl derivative 66 upon treatment with excess of 30% hydrogen peroxide gave the sulfone N-oxide 71.

NMR spectral analysis was indeed very useful in the elucidation of the structure of the sulfones. For isoxazole 68, the hydrogen peri to the sulfide function [H(6)] appeared as a multiplet at δ 7.10-7.40 along

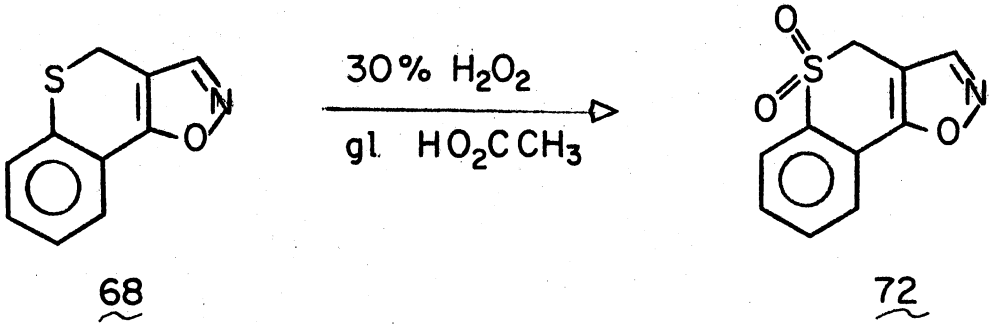


68

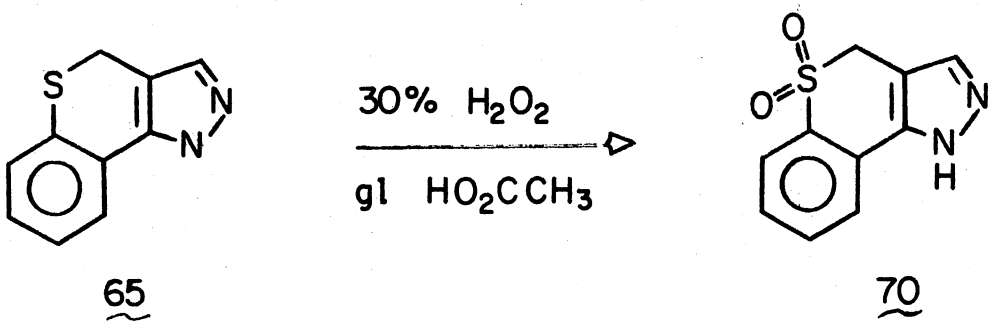


72

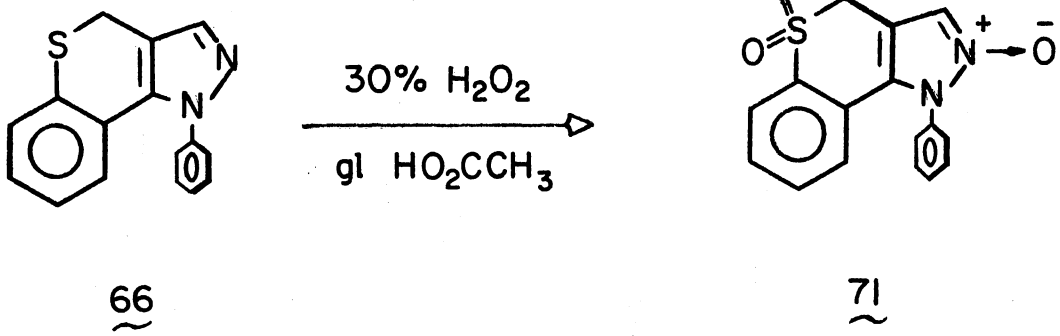
with two other aromatic hydrogens. Proton H(9) appeared as a multiplet at 7.60-7.80 ppm. Upon conversion of the sulfide to the corresponding sulfone 72, the peri proton [H(6)] was shifted downfield (δ 7.80-8.10) and appeared as a multiplet along with the H(9) proton. The methylene



68 72



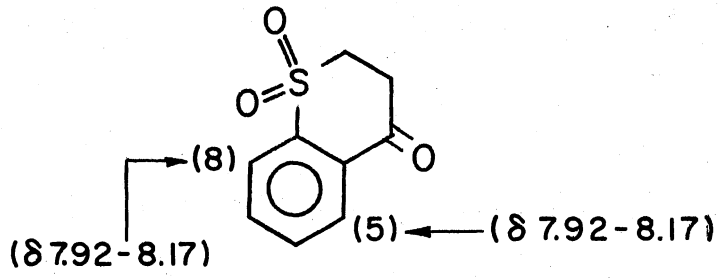
65 70



66 71

Scheme 13

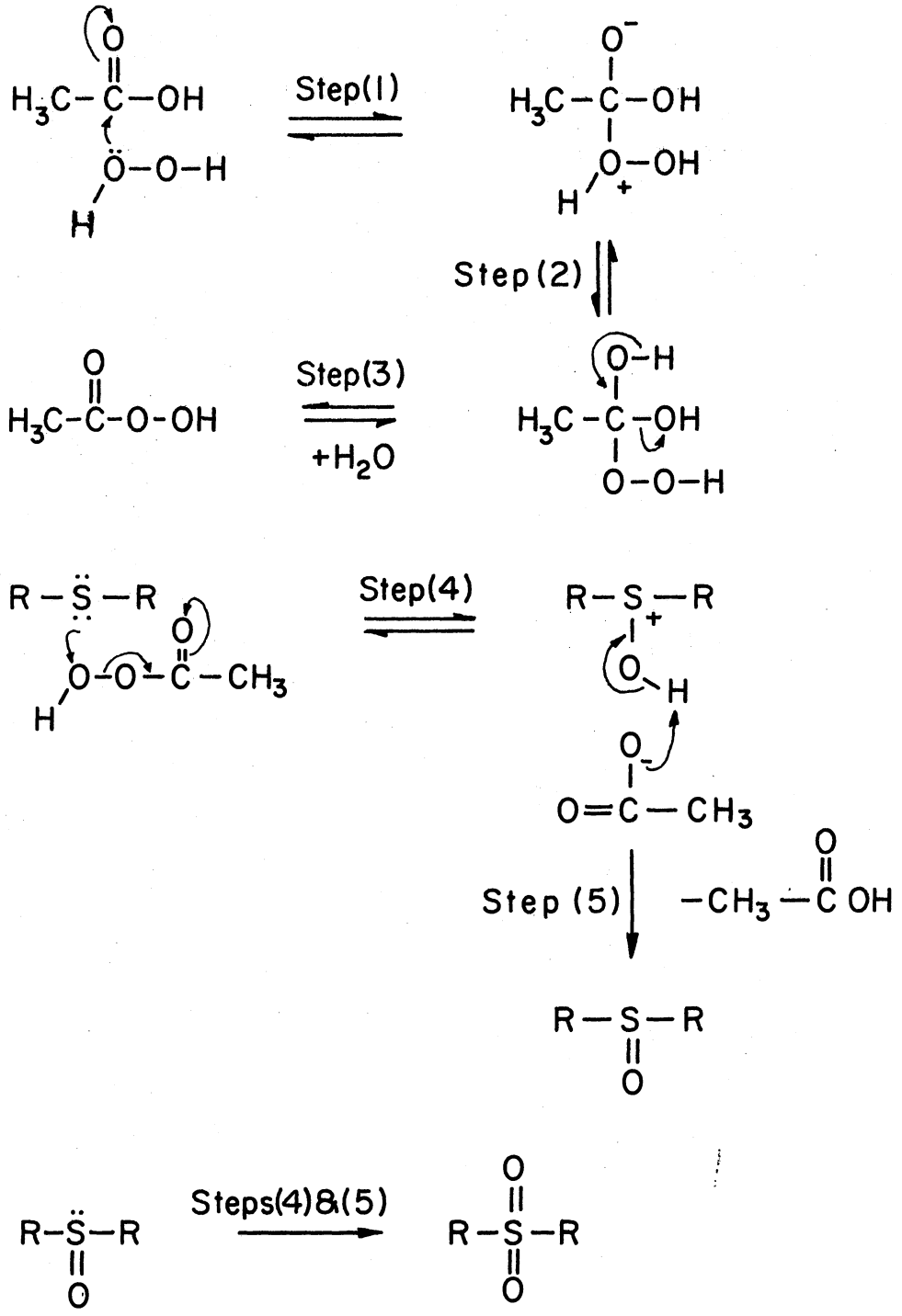
proton H(4) experienced 0.43 ppm paramagnetic shift [from δ 4.00 (68) to δ 4.43 (72)] when the sulfide was oxidized to the sulfone. A similar chemical shift pattern was observed in other cases (65 → 70 and 66 → 71). It was difficult to identify the proton peri to the carbonyl function in the sulfone 73 derived from thiochroman-4-one because of a presumably



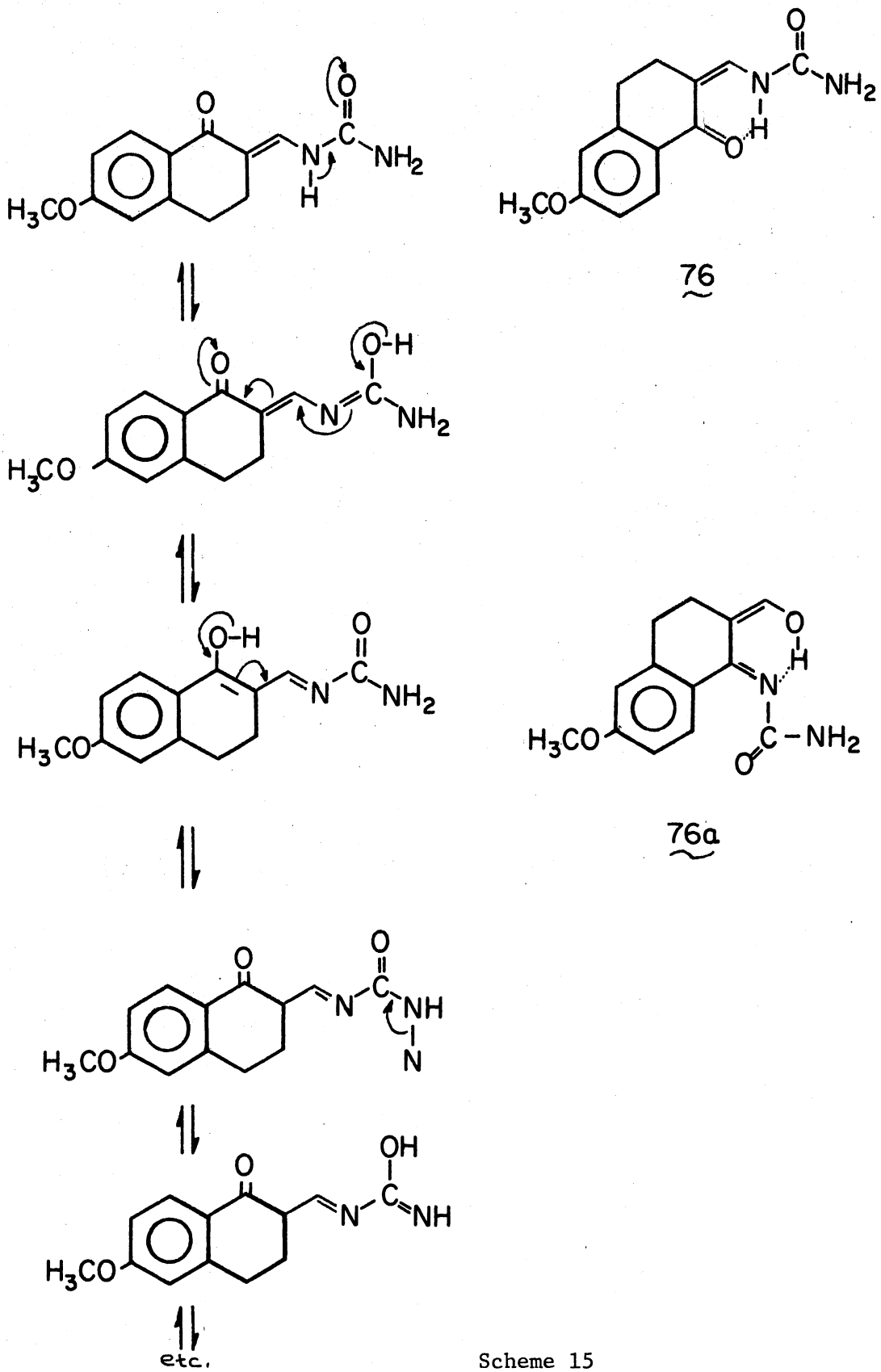
73

equivalent influence of the sulfone group on the proton in position 8. These two protons H(5) and H(8) gave, therefore, superimposed signals in the form of a multiplet at δ 7.92-8.17. Actually the proton at C(5) previously appeared as a doublet at 8.06 in 90 [before oxidation to sulfone (73)]. One plausible mechanism for the formation of sulfones, such as 73, is given in Scheme 14.

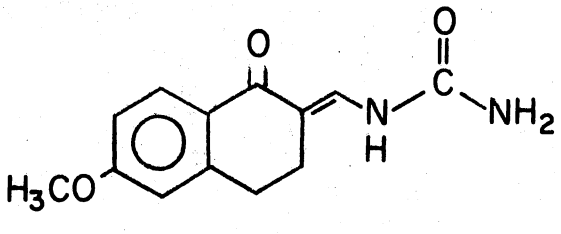
Condensation between the hydroxymethylene group and an amide function of urea or thiourea led to the formation of compounds 76, 77, and 78. This is analogous to the formation of isoxazoles (in acetic acid medium) (Scheme 15, page 87). Careful NMR and mass spectral analyses support the structures; but this does not supply unequivocal evidence to eliminate compound 76a. The presence of various tautomeric forms explained in Scheme 15 were evident from the NMR data (Table XI). Thiourea derivatives 77 and 78 also exhibited similar tautomerism as indicated by the NMR analysis (see Table XI).



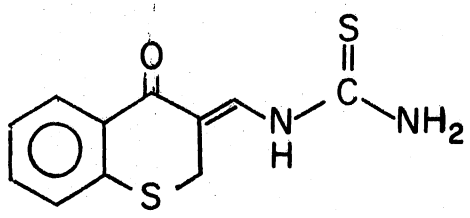
Scheme 14



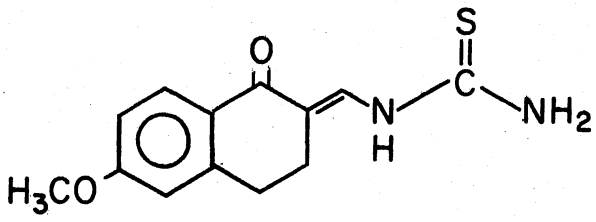
Scheme 15



76

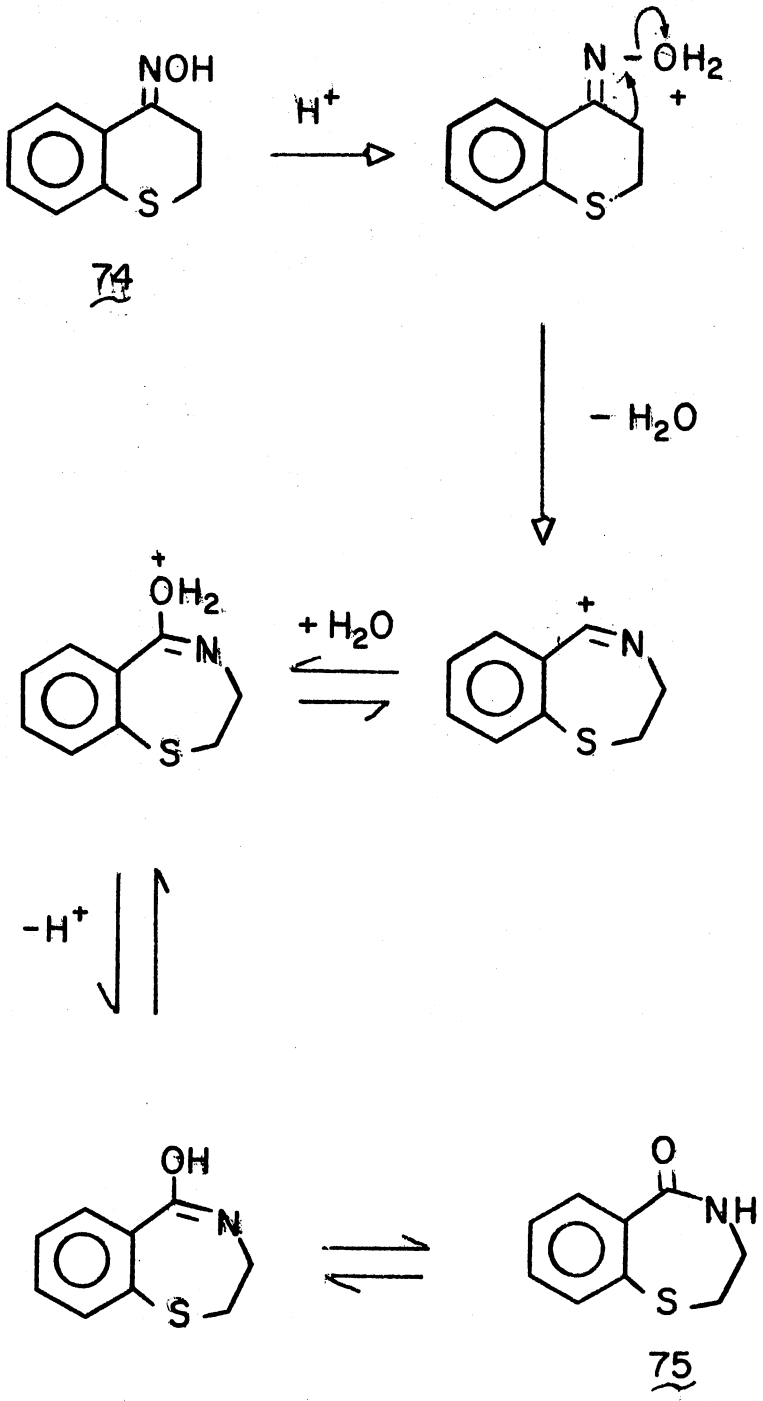


78



77

Interestingly, a fortuitous discovery was made in attempts to characterize thiochroman-4-one 90. Oximation of thiochroman-4-one occurred with hydroxylamine. The oxime 74 (m.p. 98-100°C), on standing for one hour in an amber-colored bottle, changed from a white crystalline solid to a red waxy solid. The NMR spectrum indicated formation of a lactam. This change was again noted two days after a freshly prepared sample of the oxime had been dissolved in $DCCl_3$ (for NMR analysis). The trace of acidic protons known to be present in commercial $HCCl_3$ ($DCCl_3$ contains a minute amount of $HCCl_3$) might have catalyzed the Beckmann type rearrangement. This proved interesting and suggested caution since the conversion of lactam 75 was as rapid even in a very pure, spectroscopic grade solvent that the identity of the oxime was difficult to confirm via NMR analysis. A reasonable mechanism is proposed in Scheme 16.

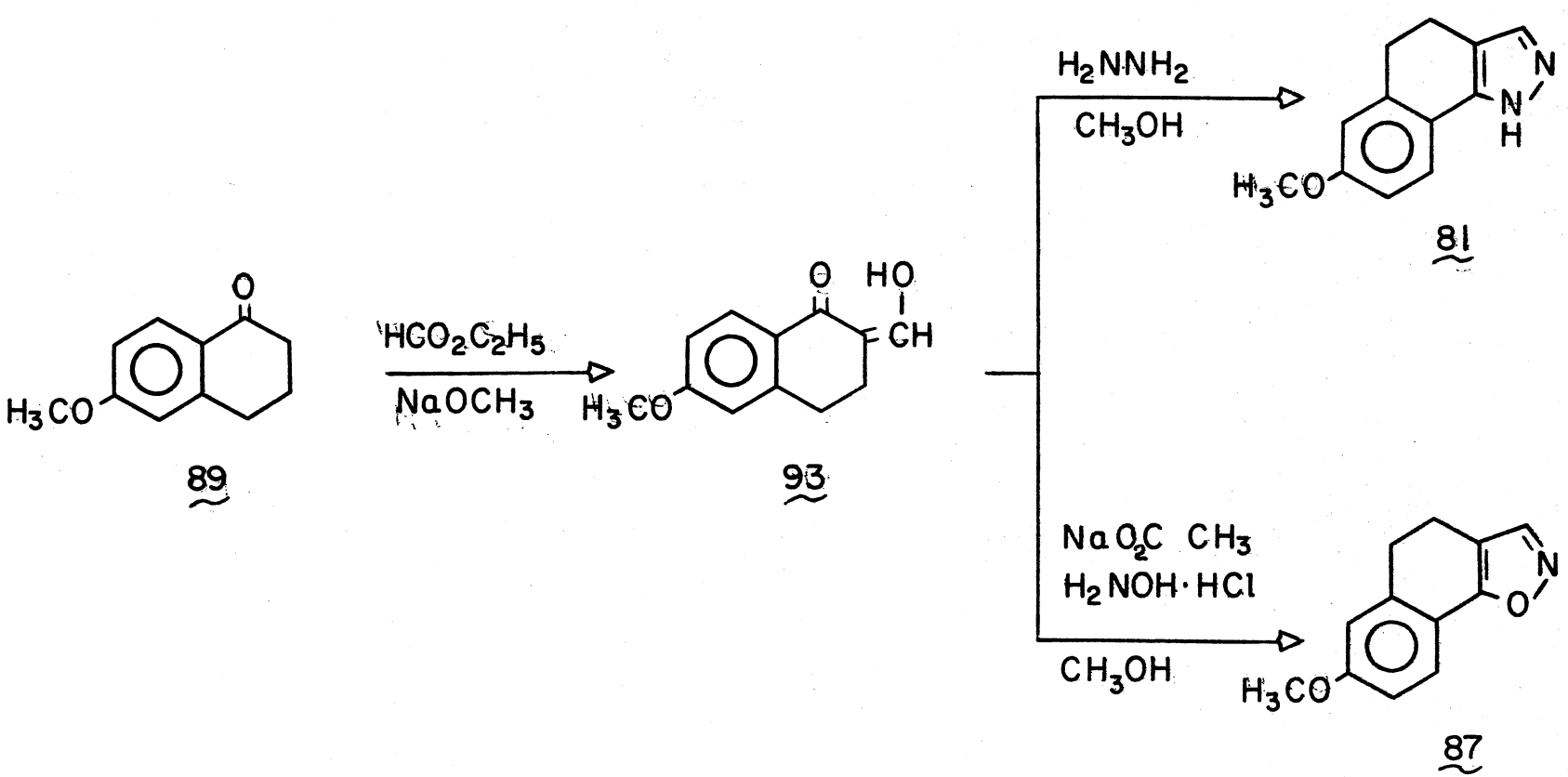


Scheme 16

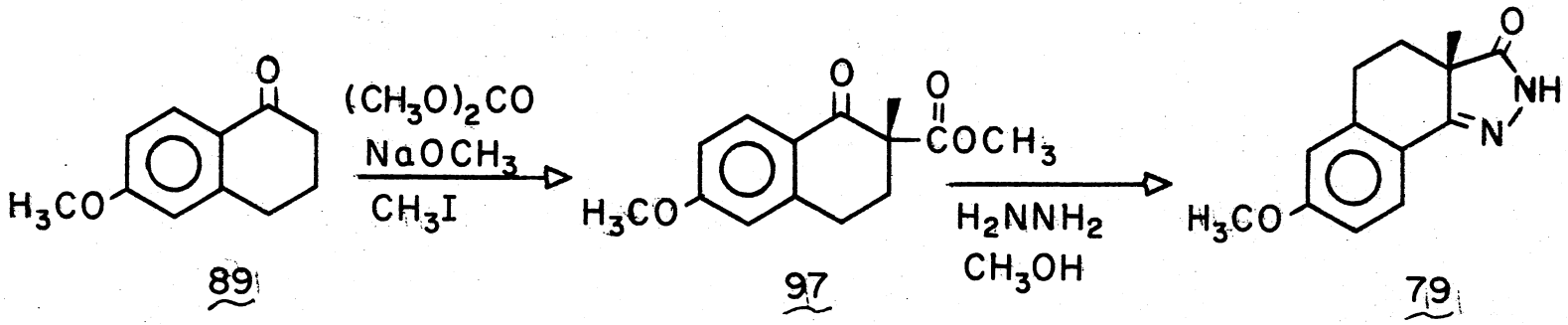
A Study of the Spectra and Acidity of 4,5-Dihydro-1H-benz[g]indazol-7-ol (82) and 2,3a,4,5-Tetrahydro-7-hydroxy-3a-methyl-3H-benz(g)-indazol-3-one (80)

6-Methoxytetralone (89) was condensed to give the hydroxymethylene ketone 93 which was then treated with hydrazine to yield the methoxy pyrazole 81; and which, on further boiling with 48% hydrobromic acid, produced the desired hydroxy pyrazole compound 82[191] (Scheme 17). Hydroxypyrazolone 80 was realized by the condensation of the α-keto ester 97 with hydrazine followed by treatment with 48% hydrobromic acid (Scheme 18). The structures of both methoxy- and hydroxypyrazolones, 79 and 80, respectively, were confirmed by elemental analysis as well as PMR spectral data. PMR analysis of the hydroxypyrazolone 80 in DMSO-d_6 revealed signals at δ 1.18 (CH_3, 3H), 2.52 (NH and OH, 2H), 1.68-2.06 (CH_2, 2H), 2.84-3.12 (CH_2, 2H) and 6.68-7.52 (ArH, 3H). This is to be compared with the spectrum of methoxy pyrazole 79, having signals (in pyridine-d_5) at δ 1.31 (CH_3, 3H), 1.62 (NH, 1H), 1.78-2.12 (CH_2, 2H), 2.76-3.08 (CH_2, 2H), 3.68 (OCH_3, 3H) and 6.77-7.94 (ArH, 3H). Deuterium oxide exchanged the protons in the compound 80 at δ 2.52 and thus confirmed the assignment of acidic protons.

It was recently found[60] that the hydroxypyrazole 82 inhibited growth of *Bacillus subtilis W23* and *Escherichia coli*. The possibility of tautomerism in both compounds 80 and 82 exists, a situation documented in simpler systems.[205] A UV spectral analysis of pyrazole 82 in water was performed over the pH range 3.2-9.6. It was anticipated

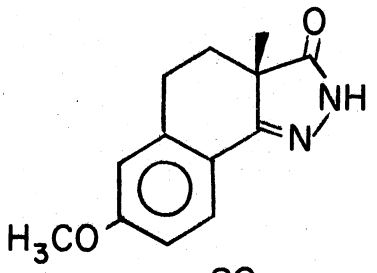

O
H3CO
89
HCO2C2H5
NaOCH3
O HO
CH
H3CO
93
H2NNH2
CH3OH
N
N
H
H3CO
81
NaO2C CH3
H2NOH·HCl
CH3OH
N
O
H3CO
87

Scheme 17

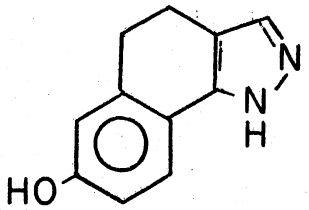


89 97 79

Scheme 18



80



82

that introduction of the carbonyl group in the pyrazole ring (i.e., in compound 80) would increase the acidity of the hydrogen on nitrogen. Such was indicated by the large increase in ε_{max} at 335 nm at pH 9.6. The absorption maxima, ε_{max}, and pH values are given for compounds 80 and 82 (Tables XII and XIII, respectively).

The msot significant observation in the spectrum of 82 was the absorption at 272.5 nm at pH 3.2 which disappeared at pH 9.6 with the appearance of a strong absorption at 280 nm and concomitant loss of shoulder at 294 nm. It has been noted previously that formation of a phenoxide ion can result in a bathochromic shift from 270 to 280 nm in water.[213,232] Considering the change at pH 9.6 and pH 3.2, the blue shift could result from hydrogen bonding, which is known to lower the energy of the n orbital.[213,232] One might expect this absorption peak (at pH 3.2) to be found at a shorter wave length. However, nitrogen atoms present in the compound under investigation are potential chromophores which could be effective in shifting the absorption toward the near visible; hence the peak's position may be justified. An additional absorption (294 nm) identified (at pH 3.2 and pH 6.0) in the form of a "shoulder" may be due to n-π* transitions of the nitrogen electrons.[213] Likewise, the bands at 205 and 207 nm are probably due to π-π* transitions in the benzene system.[232]

TABLE XII

ULTRAVIOLET ABSORPTION DATA FOR COMPOUND 80
IN AQUEOUS MEDIUM AT 10^{-5} M

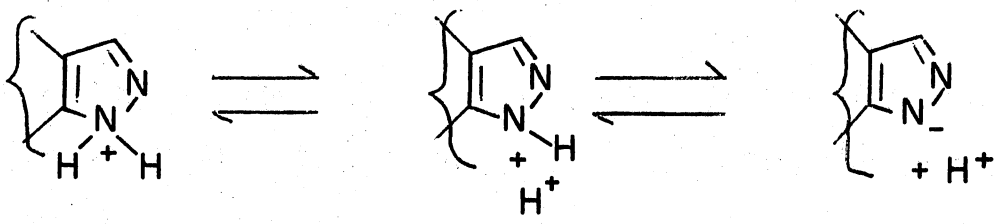
pH	Wavelength (nm)	ε_{max}
3.7	202	14,664
	225	7,332
	300	13,254
7.5	202	14,946
	226	7,050
	307	12,126
9.6	206	13,254
	273	6,486
	335	18,612

TABLE XIII

ULTRAVIOLET ABSORPTION DATA FOR COMPOUND 82
IN AQUEOUS MEDIUM AT 10^{-5} M

pH	Wavelength (nm)	ε_{max}
3.20	205	5,803
	272.5	3,839
	294 (shoulder)	2,366
6.0	205	6,607
	270	4,286
	294 (shoulder)	1,696
9.6	207	5,803
	280	3,839

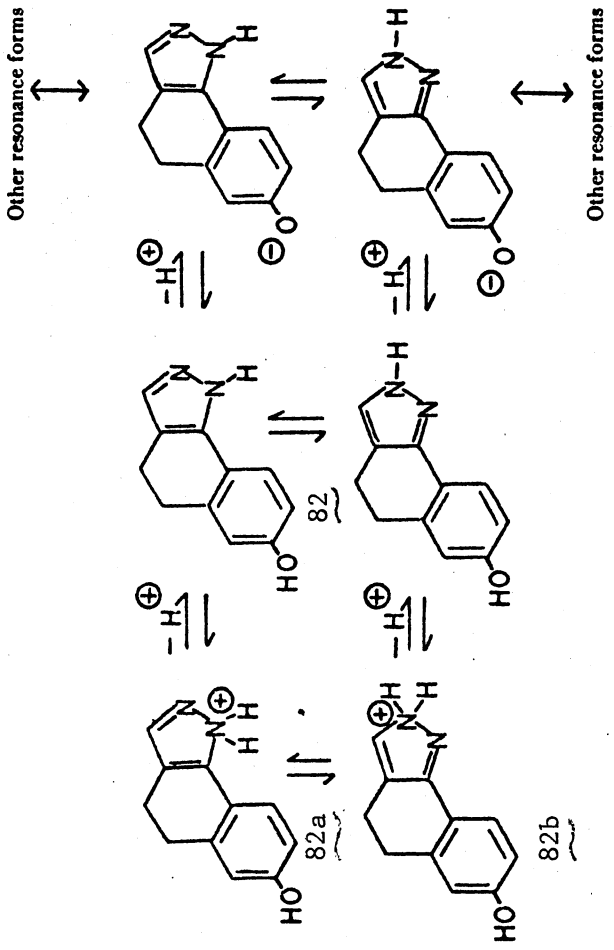
Titrations of aqueous solutions of 82 with standard, aqueous sodium hydroxide and standard aqueous hydrochloric acid revealed two inflections with pK_a values determined to be at 9.1 and 3.7. Pyrazole has a reported pK_a of 2.5[181] and phenol a value of 9.89.[181] Thus, a first approximation would assign the value of 3.7 to the ionization process involving the proton on nitrogen in cation 82a (or cation 82b) (Scheme



19). The pK value of 9.1 value is likely due to the ionization of the phenolic proton. The remaining proton on nitrogen can scarcely be involved since pyrazole itself is reported to have a pK_a at about 14.[3]

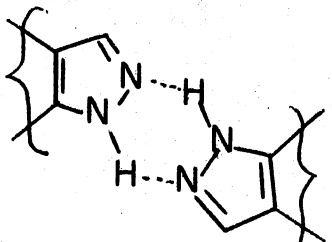
Unfortunately, the UV spectrum of a close, structurally-related model system for the pyrazole 82 could not be found in the literature. However, PMR analysis in pyridine-d_5 revealed a signal at δ 12.4 (NH or OH, 2H) indicating two acidic protons [other protons are at δ 2.78 (CH_2-CH_2, 4H) and 6.78-8.12 (ArH and -C=CH, 4H)]. Thus, together these data support the structure but do not give unequivocal evidence to eliminate the presence of tautomer 82a or 82b in neutral solution.

If dimer formation via H-bonding occurs, as was previously indicated from an infrared analysis of pyrazole,[3,6] the difference in steric requirements for the dimers would probably not be as great as estimated from Courtauld models. Also, PMR studies on a series of pyrazoles support the presence of dimers of pyrazole in $DCCl_3$ and

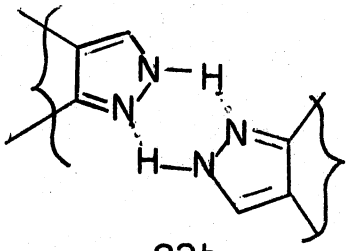


Scheme 19

and CF_3CO_2H.[92] Intuitively, pyrazole 82b in water might be less stable than pyrazole 82a since both double bonds in the smallest ring of 82b are exocyclic to a six-membered ring.

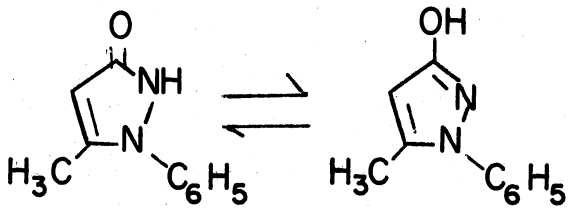


82a



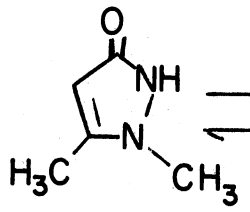
82b

As part of a study of heterosteroids and model systems, the synthesis of pyrazolone 80 provides a carbonyl group in the appropriate position of the smaller ring (for possible improved biological activity since the heterosteroids are related to equilenin) which could promote enolization. Although several careful studies have been made of the tautomerism in various pyrazolones,[149,222] the closest simple model systems for 80 are 102 and 103, which were reported to exist in both

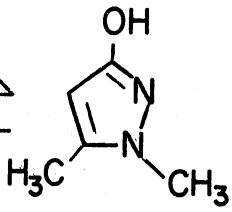


102

102a

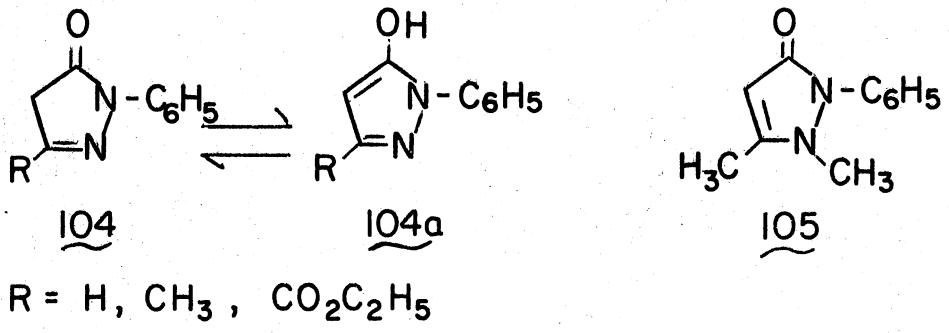


103



103a

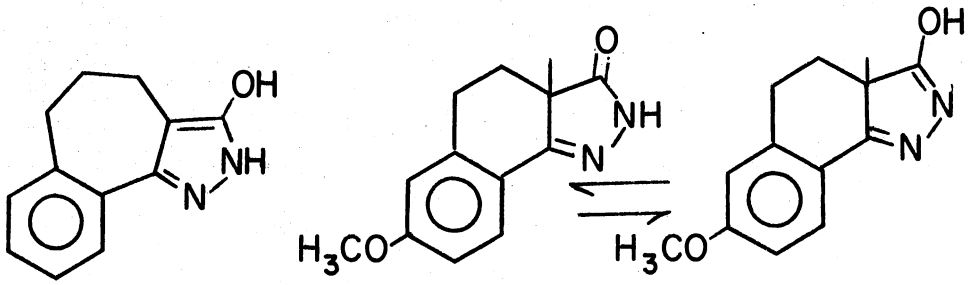
enol and oxo forms in water by IR, UV and PMR analysis. Acidity measurements in water gave pK_a value of 8.23 and 8.91 for 102 and 103, respectively.[150] An order of stability of tautomers in water was given in a later paper as shown below.[149] ^{13}CMR confirmed the existence of the enol form 104a rather than oxo form 104, while nonenolizable 105



104 104a 105

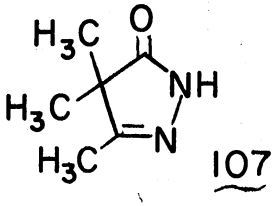
R = H, CH_3, $CO_2C_2H_5$

showed only a carbonyl carbon atom in DMSO as expected.[237] A recent report tentatively suggests that compound 106 exists in the enol form in DMSO (PMR study).[78] In a polar medium, 106 was not expected to be a good candidate on the basis of the stability studies of pyrazolones reported earlier,[149] although a broad two-proton signal was observed in the PMR spectrum for 106 at δ 10.65 (NH or OH) (pK_a = 9.69).[78]

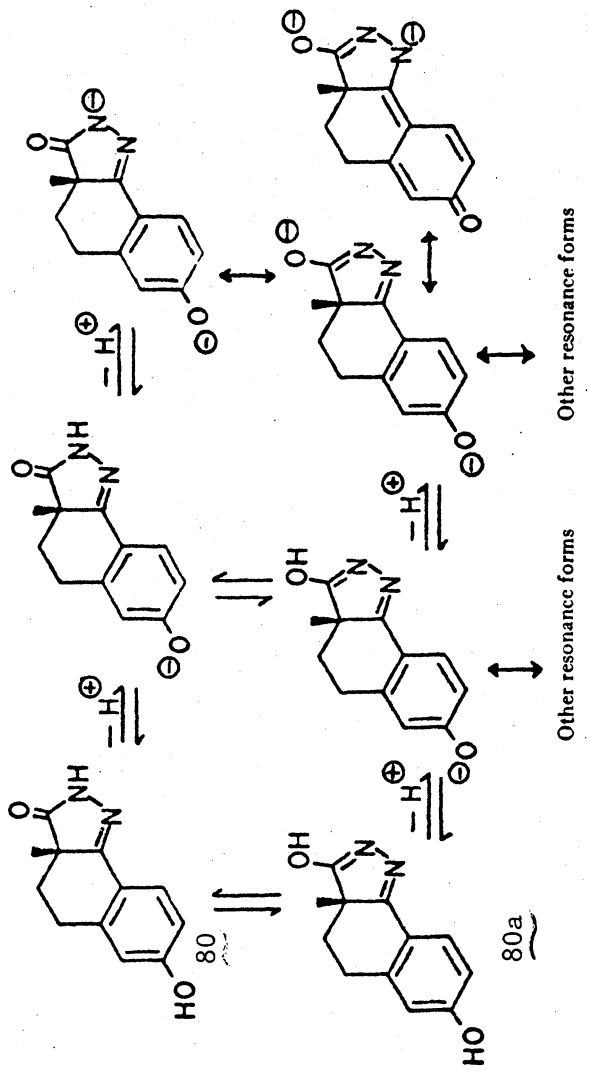


106 79 79a

If we consider 80 and 80a in our study (Scheme 20), IR analysis (solid state) showed a strong peak at 1639 cm^{-1}. [This is a dramatic shift compared to the ether precursor 79, which had $\nu_{C=O}$ at 1686 cm.] The latter supports the oxo form 79 rather than the enol form 79a.



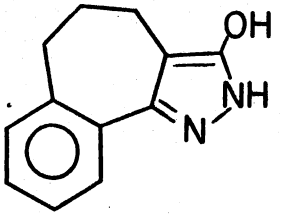
107



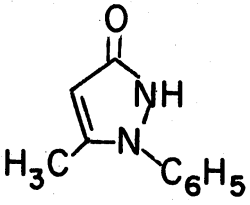
Scheme 20

There was also a broad band at 2824-3125 cm^{-1}, with maxima at 2898, 3030, and 3125 cm^{-1}, which support the presence of aliphatic and aromatic C-H bonds in 79. For comparison, compound 107 is reported[149] to have $\nu_{C=O}$ at 1715 cm^{-1} in $HCCl_3$ and a doublet in CCl_4 at $\nu_{C=O}$ at 1734 and 1718 cm^{-1}. Since 80 has $\nu_{C=O}$ at 1639 cm^{-1} (KBr disc), perhaps H-bonding dimers or higher-order polymeric-type structures exist in the solid state as suggested for certain pyrazolones.[149,150] Credence is lent to this tentative supposition by the high melting point (328°, with decomposition) of 80. Mass spectral analysis gave the correct m/e (216) for M^+, but dissociation of a dimer could have occurred in or near the ion source (250°) prior to decomposition.

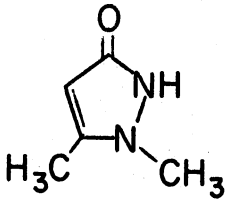
Titration (in water) of 80 (or 80a) revealed a pK_a of 8.7, which is surprisingly between that of 106 (9.69)[237] and 102 (8.23) and close to that of 103 (8.91).[150] Recall that the latter is believed to exist as



106



102

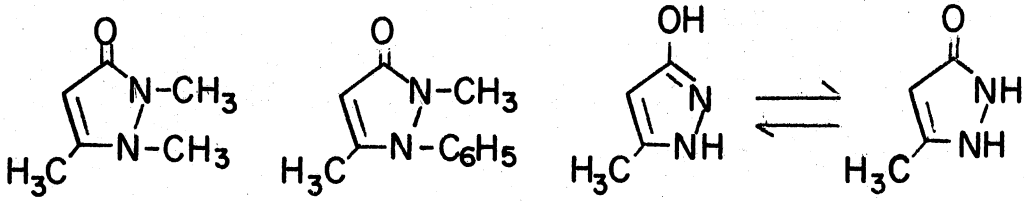


103

equilibrium mixture (page 97) 102 ⇌ 102a and 103 ⇌ 103a.[150] A tentative assumption is that the pK_a of 8.7 for 80 represents an average value not only for a tautomeric mixture (H on either of the two N atoms) but for the phenolic form also.

The ultraviolet spectrum (in triply distilled water) of the pyrazolone 80 gave absorption maxima of high intensity as shown in Table XIII. Good correlation of these intensities and maxima with data

for known close model systems is difficult because of the additional absorption of the arene portion of 80. However, the intensities for 108[150] and 109[150] contrast somewhat with those for 110.[149] In aqeuous media, a predominance of 110a (∿ 80%) was suggested.[149]



108 109 110 110a

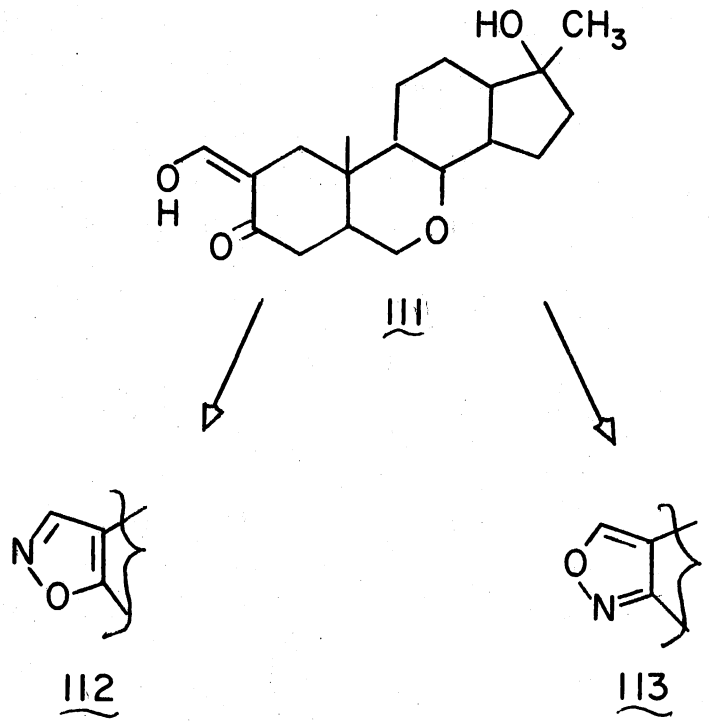
In 80, the maxima at λ = 225 nm (pH = 3.7 and at λ = 226 nm (pH = 7.5) have intensities close to that of 110a at λ = 238 nm (pH = 5.2) as illustrated and are probably comparable thereto on the reasonable assumption the maxima are due to π-π* transitions. Since position 3a in 80 does not possess an enolizable proton, exact comparison of these tautomeric systems must be treated cautiously, however. This is reinforced by the recent work showing 104a the preferred tautomer rather than 104. Nevertheless, in 80 there is no driving force to favor 80a as there is in 104a ⇌ 104 where a phenyl ring on nitrogen might provide such driving force to favor 104a. Thus, taken on the whole, the evidence suggests tautomer 80 predominates over 80a in the solid state as well as in aqueous solution.

Structural Determination of Two Isomeric Isoxazoles via ^{13}CMR Analysis

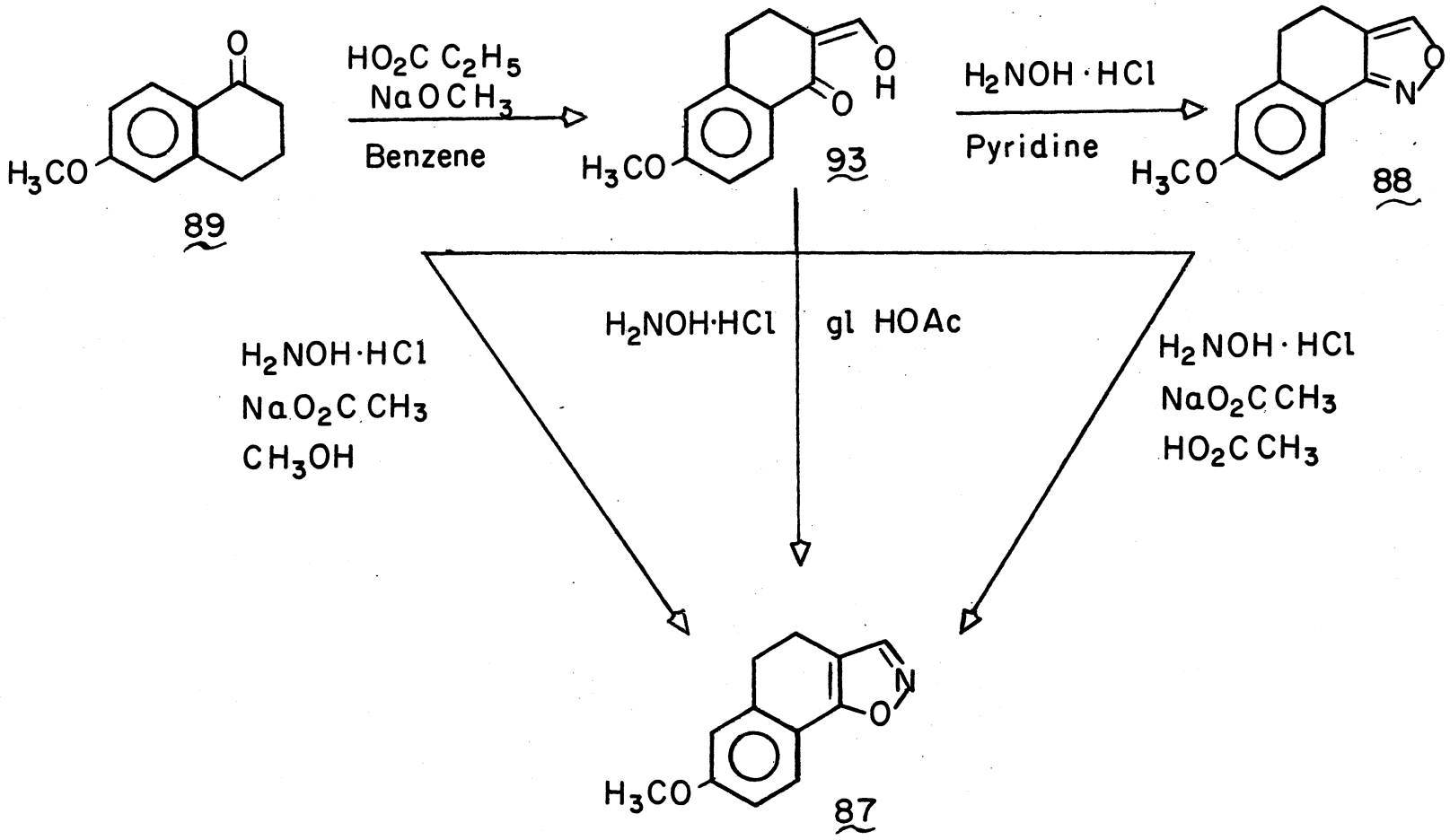
Two previously known isomeric isoxazoles 87 and 88 were synthesized from α-hydroxymethylene-6-methoxy-1-tetralone (93) and hydroxylamine

hydrochloride. The possibility exists that these two isomeric isoxazoles may be distinguished by ^{13}CMR spectral analysis. Four sets of reaction conditions (Scheme 12) could produce one or both isomers. Since disagreement is still found in the literature[112,183] as to the formation and identity of the two isomers (and in view of much NMR data supporting two structures), a critical ^{13}CMR analysis seemed a plausible means to solve this problem.

According to Guthrie and co-workers,[112] reaction of the hydroxymethylene compound 111 with $H_2NOH \cdot HCl$ in acetic acid containing sodium acetate afforded the [2,3-d]isoxazole 112. Supposedly, $H_2NOH \cdot HCl$ in



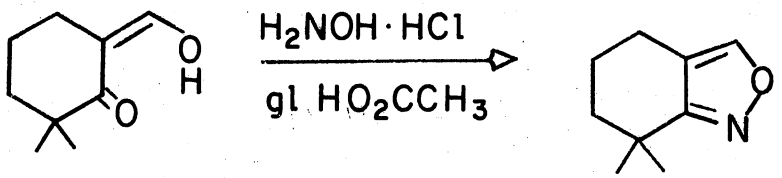
pyridine yielded a mixture of the isomeric isoxazoles from which the [3,2-c]isoxazole 113 was isolated by the previously prescribed method.[173,175,251] Johnson and Shelberg[141] (and more recently Jacquier and co-workers[138]) found isoxazole formation in acetic acid medium


O
H3CO
89
HO2C C2H5
NaOCH3
Benzene
OH
O
H3CO
93
H2NOH·HCl
Pyridine
O
N
H3CO
88
H2NOH·HCl
NaO2C CH3
CH3OH
H2NOH·HCl
gl HOAc
H2NOH·HCl
NaO2C CH3
HO2C CH3
N
O
H3CO
87

Scheme 12

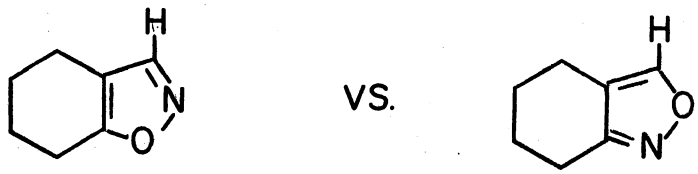
gave the [2,1-d]isomer. If the reaction was carried out in pyridine, the isomeric [1,2-c]isoxazole resulted.[112] However, no x-ray data are available.

In contrast to an analogous example in the literature,[183] (according to Meyer and co-workers[183]) if the oximation was performed directly on the hydroxymethylene ketone 114 by the general procedure (using hydroxylamine hydrochloride in acetic acid), isomeric [1,2-c]isoxazole 115 was the major product, accompanied by the other isomer (34%). Again, x-ray data are lacking.

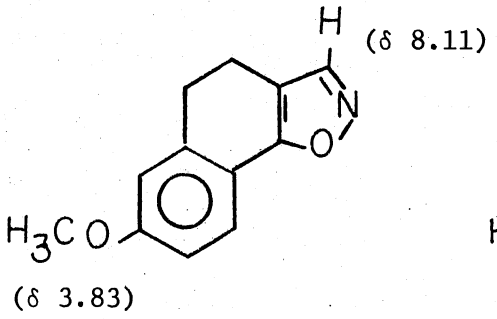


114 115

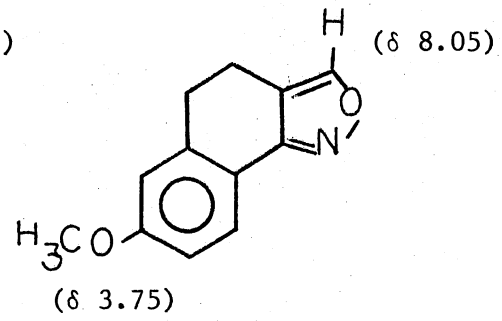
In our investigation (Scheme 12), PMR analysis of the products obtained from methods A, B and C (see Experimental) revealed them to be identical. When the reaction was carried out in pyridine (method D), isomeric [1,2-c]isoxazole resulted. These findings are in good agreement with reports in the literature[19] for somewhat similar systems. For example, a difference in chemical shift of 0.05 ppm was observed for the



two isomers shown.[19] We found a difference of 0.06 ppm between the chemical shifts of the indicated heterocyclic protons of isomers 87 and 88.



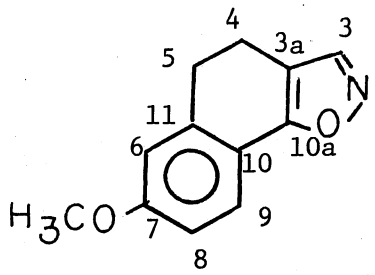
87



88

The primary question that arises is whether this small chemical shift difference derived from PMR analysis can be accepted with confidence. It may be noted that a larger difference in chemical shifts (0.08 ppm) occurred in the protons of the methoxy groups of the two isomeric isoxazoles which is not easily rationalized. Since PMR data cannot unequivocally substantiate one structure, a ^{13}CMR analysis was considered more useful because of the enormous sensitivity of ^{13}C chemical shifts to structural changes.[38,77,108] Another interesting property of ^{13}CMR spectra is that each carbon atom of the skeleton and its coupling with any attached group (NMR active) may ordinarily be individually examined.

Since the compounds 87 and 88 differ mainly on the location of O and N atoms, the bonded carbons should be affected more, i.e., C(10a) and C(3) should have a different δ value in 87 compared to 88. It was

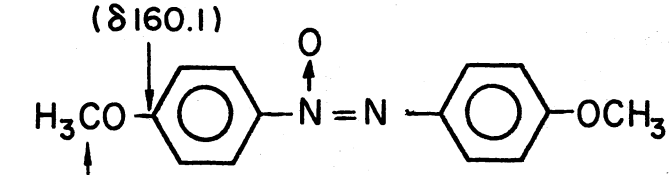


87

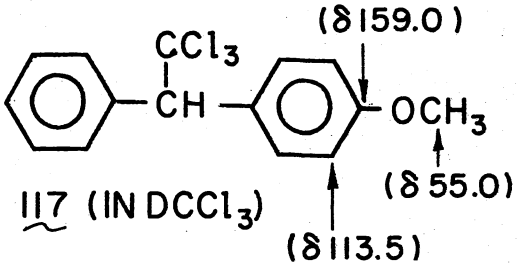
gratifying to find a major change in the chemical shifts with respect to these positions. Assignments were made by comparison with model systems 116, 117, 118, 119, and 120.

It has been found that methoxy carbon at C(7) can readily be identified along with the aromatic carbon atom C(7) itself, from similarly documented model compounds 116, 117 and 120. Thus δ 55.2 (for both compounds 87 and 88) was assigned to the methoxy carbon. Signals at δ 161.1 and δ 161.4 were assigned to C(7) in compounds 87 and 88, respectively. The signals of δ 112.4 and δ 114.9 (for 87) were attributed to C(6) and C(8), although this assignment is tentative. Similarly δ 113 and δ 114.8 were assigned to C(6) and C(8) of isoxazole 88 (compare model compound 120).

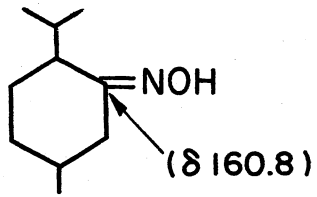
The resonance signals at δ 110.8 (for 87) and δ 114.2 (for 88) may be assigned to C(9). This must be considered tentative also. The signals at δ 139.2 (for 87) and δ 140.6 (for 88) can be assigned to C(11) (compare model compound 120). The difference in chemical shifts for C(10) may be ascribed to differences in strain.[107,139,219,224] The upfield shifts resulting from steric interactions with the c-ring is evidenced from the observed shift trends (δ 123.5 for 87 and δ 126.5 for 88). Again the 3 ppm downfield shift for 88 may be due to lesser degree of strain in comparison to 87, where a larger oxygen atom occupies the place of a nitrogen atom. Carbons C(5), C(4) and C(3a) can readily be identified by reference to 119 and 120. In both cases upfield shifts experienced by C(4) may result from steric interactions with a π-electron cloud which was absent in both model compounds 119 and 120. As expected, two resonance signals underwent substantial changes and may thus be easily assigned to the carbons C(10a) and C(3). The carbon



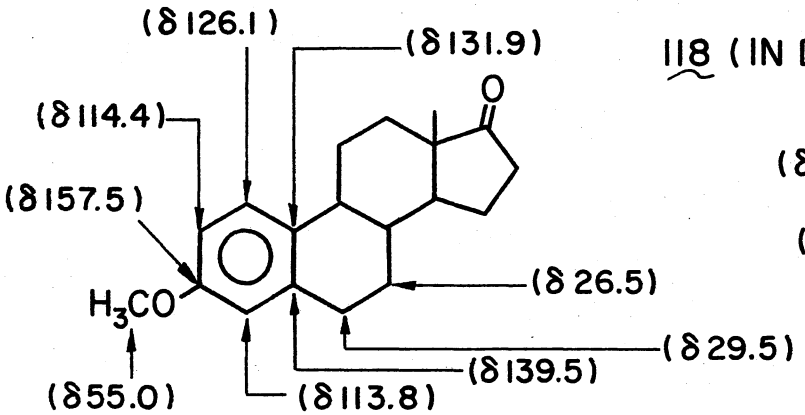
116 (IN $DCCl_3$)



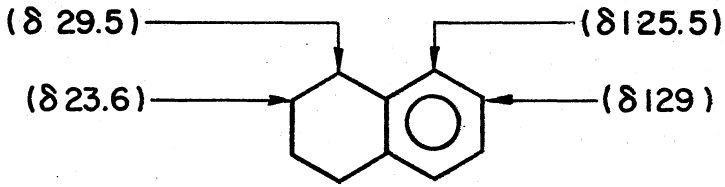
117 (IN $DCCl_3$)



118 (IN $DCCl_3$)



120 (IN $DCCl_3$)



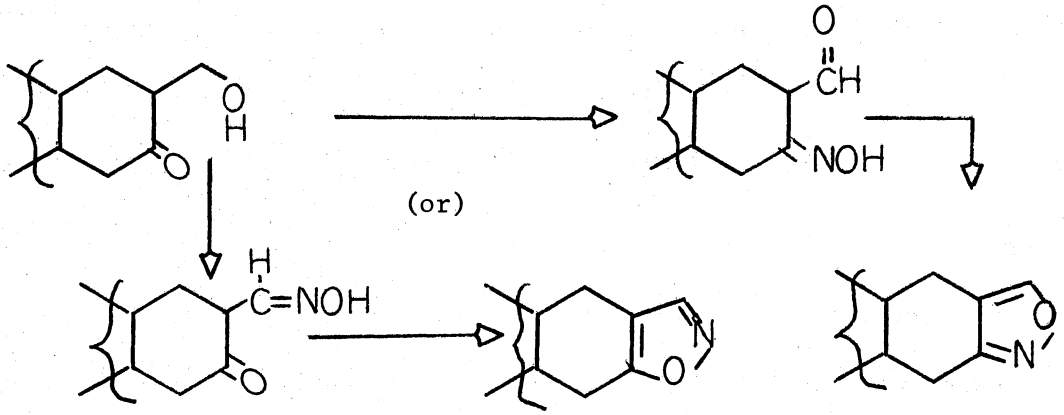
119 (IN $DCCl_3$)

which experienced the most downfield shift is likely C(10a) of compound 87 and hence the signal at δ 165.8 was assigned to C(10a) and δ 149.2 to C(3) (for 87). At C(10a) (for 87) the presence of an electronegative atom on a quaternary carbon gave rise to pronounced paramagnetic shift. For the compound 88, δ 158.5 and δ 152.5 signals may be assigned to C(10a) and C(3), respectively. Thus, C(3) of 87 can also be identified by the characteristic quadrapole broadening. C(10a) and C(3) signals for both compounds are of lower intensity owing to longer reaction times and lower nuclear Overhauser enhancements. It is interesting to note, as expected, that the methoxy carbon atom, possibly because of a larger nuclear Overhauser effect, appeared at higher intensity.

The nuclear Overhauser effect (NOE) is a by-product of proton noise decoupling (so that splitting due to spin coupling between ^{13}C and ^{1}H will be collapsed) in ^{13}CMR experiments. This process disturbs the Boltzmann distribution of the two energy levels of ^{1}H.[169a] The ^{13}C nuclei depend mainly on the ^{1}H nuclei for spin-lattice relaxation.[169a] Since the exact magnitude of the NOE depends on the nature and environment of a specific carbon atom, the integrated intensities of ^{1}H-decoupled ^{13}C resonance signals can vary in a single molecule.[169a,233a] This limits the usefulness of proton-decoupled ^{13}C spectra for quantitative analysis. Experimentally, NOE means that more radiofrequency energy will be absorbed by the ^{13}C nuclei as a result of the larger population in the lower energy level.[169a]

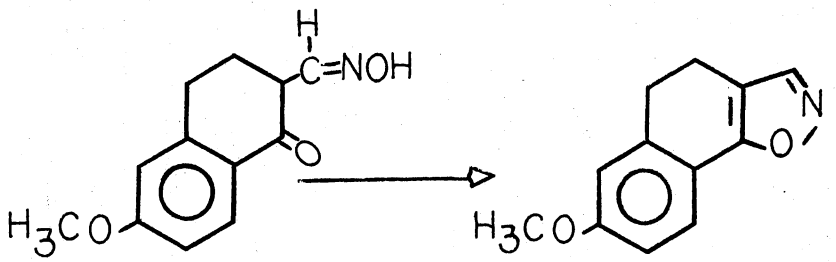
In the process of formation of the isoxazole, an oxime may be generated. The oxygen atom of the oxime, being more nucleophilic, may attack the carbonyl carbon; this would lead to isoxazole formation.

When a α-hydroxymethylene ketone is condensed with hydroxylamine hydrochloride in acetic acid, one might visualize two possible mechanisms of formation of the oximes (Scheme 21). The formation of isomeric



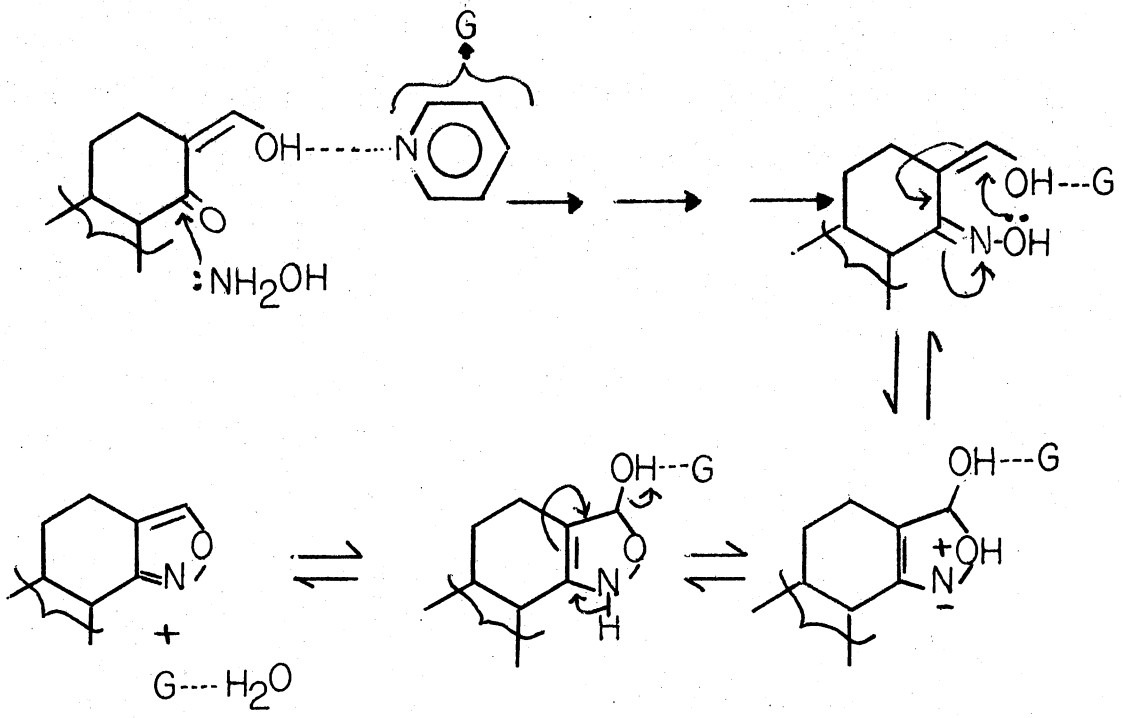
Scheme 21

isoxazoles may actually depend upon the kind of intermediate oxime formation. The compound 87 might have formed as a result of the following intermediate oxime.



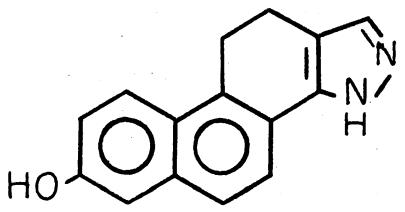
87

In pyridine, ketoxime formation may be favored by the possible coordination between hydroxyl hydrogen and pyridine as illustrated. This coordination might prevent attack of hydroxylamine at the aldehydic carbon atom (the hydroxymethylene form is in equilibrium with the aldehyde form). Therefore, the nucleophilic nitrogen may attack the carbonyl group as represented to give an isomeric isoxazole. Thus, it is now reasonable to assume that the formation of two isomeric isoxazoles 87 and 88 occurs via two different mechanistic pathways.



Molecular Complexation Studies

A number of physiologically active azasteroids have been found to alter membrane permeability[232a] in certain systems. It was reported[119] recently that the uptake of ^{14}C-uracil was specifically inhibited by 10,11-dihydro-3H-naphth[1,2-g]indazol-7-ol (24). But, surprisingly,



24

neither 5-fluorouracil nor mitomycin C (two known anticancer drugs) was potentiated by hydroxyindazole 24.[119] It was equally interesting that another anticancer drug, actinomycin D, showed an enhanced activity[119]

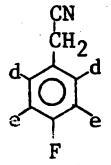
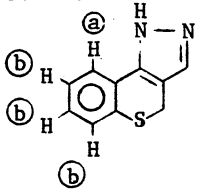
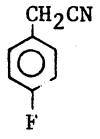
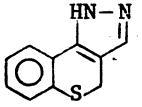
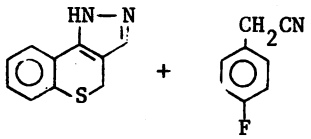
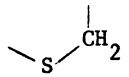
usen used in combination with hydroxyindazole 24. Since an understanding of the specific nature and physiochemical properties of molecular complexes of certain heterocycles (having strategically positioned heteroatoms) with anticancer drugs could be instructive, molecular complexes of certain pyrazoles and isoxazoles with 5-fluorouracil (anticancer drug) and with other acceptor candidates have been studied in this work. A vast literature indicates that formation of molecular complexes can be deduced from the analysis of changes in the absorption spectra of mixtures and from the NMR chemical shift difference in comparison with the spectra of individual compounds.[53a]

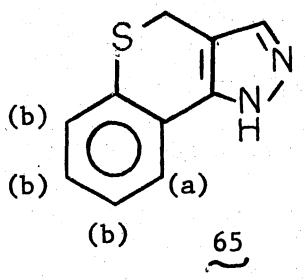
Our study with a model system included PMR chemical shifts measurements of 1,4-dihydro[1]benzothiopyrano[4,3-c]pyrazole (65) (TP) and 1,3,5-trinitrobenzene (121) (TNB) and their complexes; the results are summarized in Table XIV. As can be observed, the diamagnetic shift experienced by the ring methylene protons of the pyrazole group in the molecular complex was 0.043 ppm. The aromatic protons (3H) of trinitrobenzene also showed an upfield shift, 0.078 ppm. It is apparent from Table XIV that the ring CH proton has shifted 0.05 ppm upfield. Since the protons on N are somewhat acidic in 65, an intermolecular association could occur between 65 and another identical molecule or with 121.

The anomalous diamagnetic shift of aromatic H_a proton, in comparison to H_b protons, suggests the direct interaction of nonbonding electrons on one of the oxygen atoms of the nitro group of trinitrobenzene in the complex. It can reasonably be concluded from the observed shifts that the pyrazole system of 65 interacts notably with 1,3,5-trinitrobenzene.

TABLE XIV

CHEMICAL SHIFT CHANGES IN 1:1 COMPLEX OF 1,4-DIHYDRO[1]BENZOTHIOPYRANO-[4,3-c]PYRAZOLE AND 1,3,5-TRINITROBENZENE IN ACETONE d_6

Kinds of Proton with Respect to		Chemical Shifts in H_z			Difference in Chemical Shifts in H_z
		 uncomplexed	 uncomplexed	 complexed	
	CH		752	751.6	0.4
			400.5	399.5	1.0
	Aromatic ⓐ		786	787	1.0
	Aromatic ⓑ		723		
CH_2-CN		392		389.2	2.8
Aromatic ⓓ		725			
Aromatic ⓔ		742.8		741.8	1.0



65

In Figures 8 and 9 are shown the signals for the ring methylene protons of the pyrazole 65 and the 3 aromatic protons of 1,3,5-trinitrobenzene 121, respectively, in both the complexed and the uncomplexed systems.

The formation of a molecular complex is further substantiated by ultraviolet absorption bands recorded in Table XV.

The most significant feature in the spectrum of the molecular complex is the disappearance of the absorption peak at 245 nm and concomitant broadening of the absorption band at 230 nm with simultaneous decrease in ε_{max}. A reasonable structure consistent with the observed shift trends is given in Figure 10.

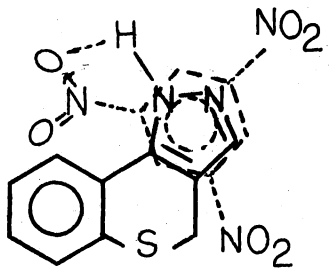


Figure 10. Schematic Representation of TP-TNB Complex

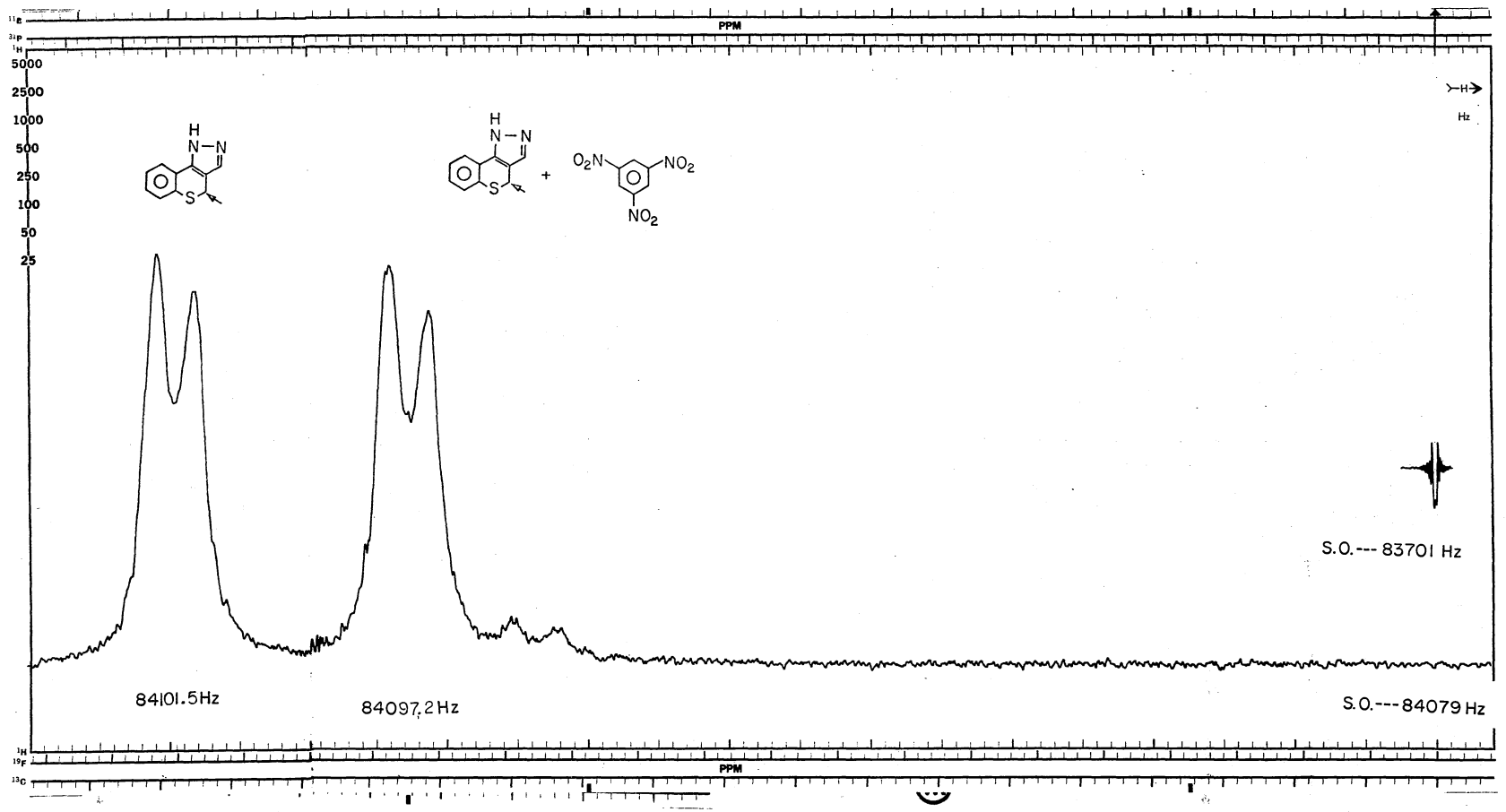


Figure 8. PMR Spectra Showing Chemical Shifts of the Ring Methylene Protons of the Pyrazole 65

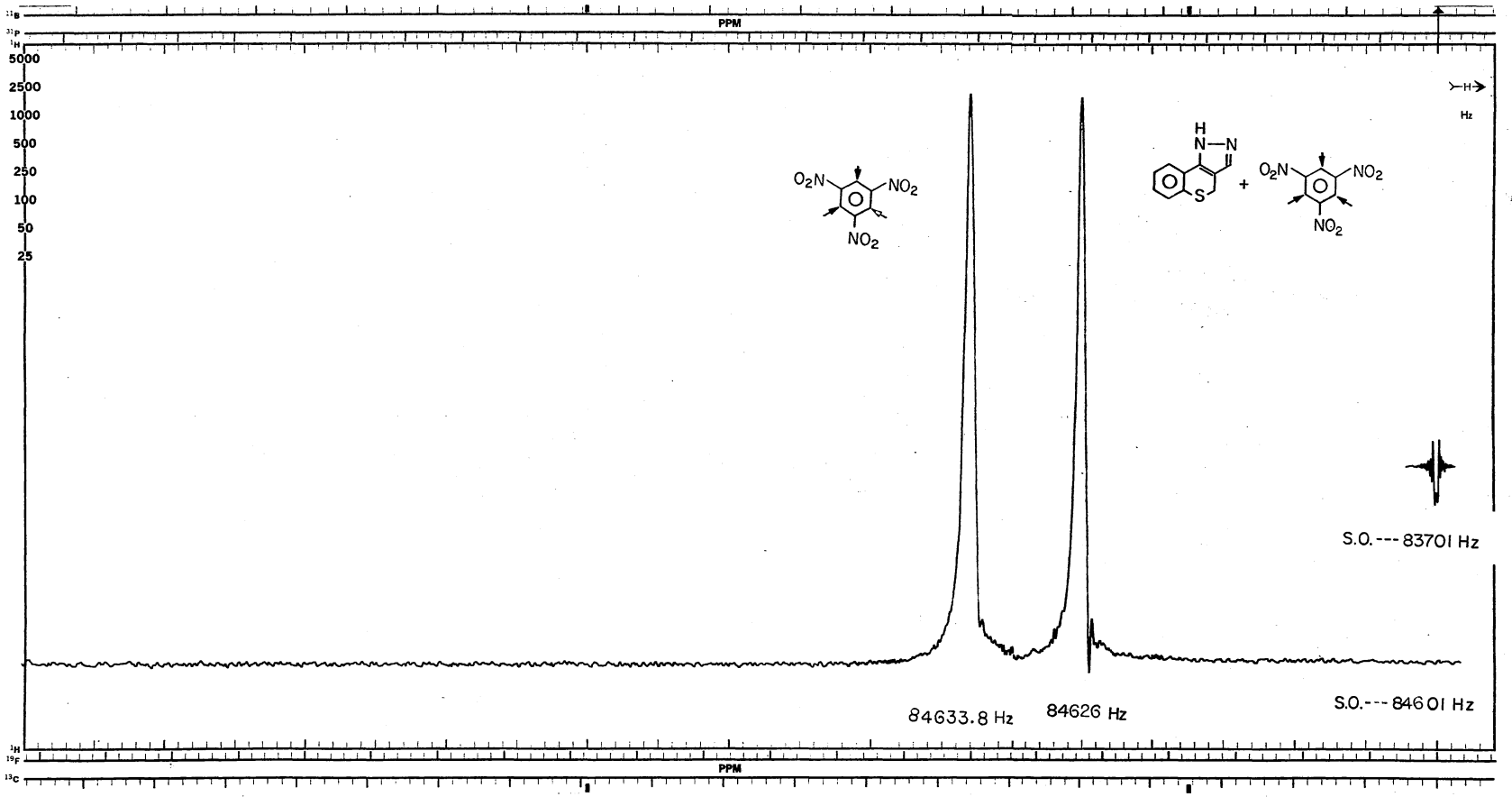
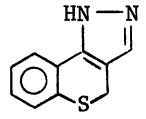
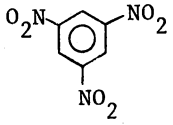
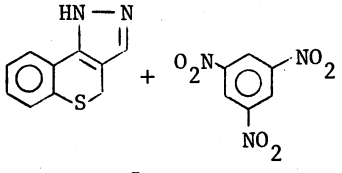


Figure 9. PMR Spectra Showing Chemical Shift of the Three Aromatic Protons of 1,3,5-Trinitrobenzene 121

TABLE XV

ULTRAVIOLET ABSORPTION DATA FOR 1,4-DIHYDRO[1]-BENZOTHIOPYRANO[4,3-c]PYRAZOLE, 1,3,5-TRINITROBENZENE AND THEIR COMPLEX IN 95% ETHANOL

Compounds	Complex	U.V. Absorption	
		Wavelength in nM	ϵ_{max}
 (9.636×10^{-5} m/l)		312 245	2700 18580
 (9.636×10^{-5} m/l)½		230	29680
	 9.63×10^{-5} m/l each diluted to 150 ml (1:1 complex)	312 230 (broadens)	1400 21810

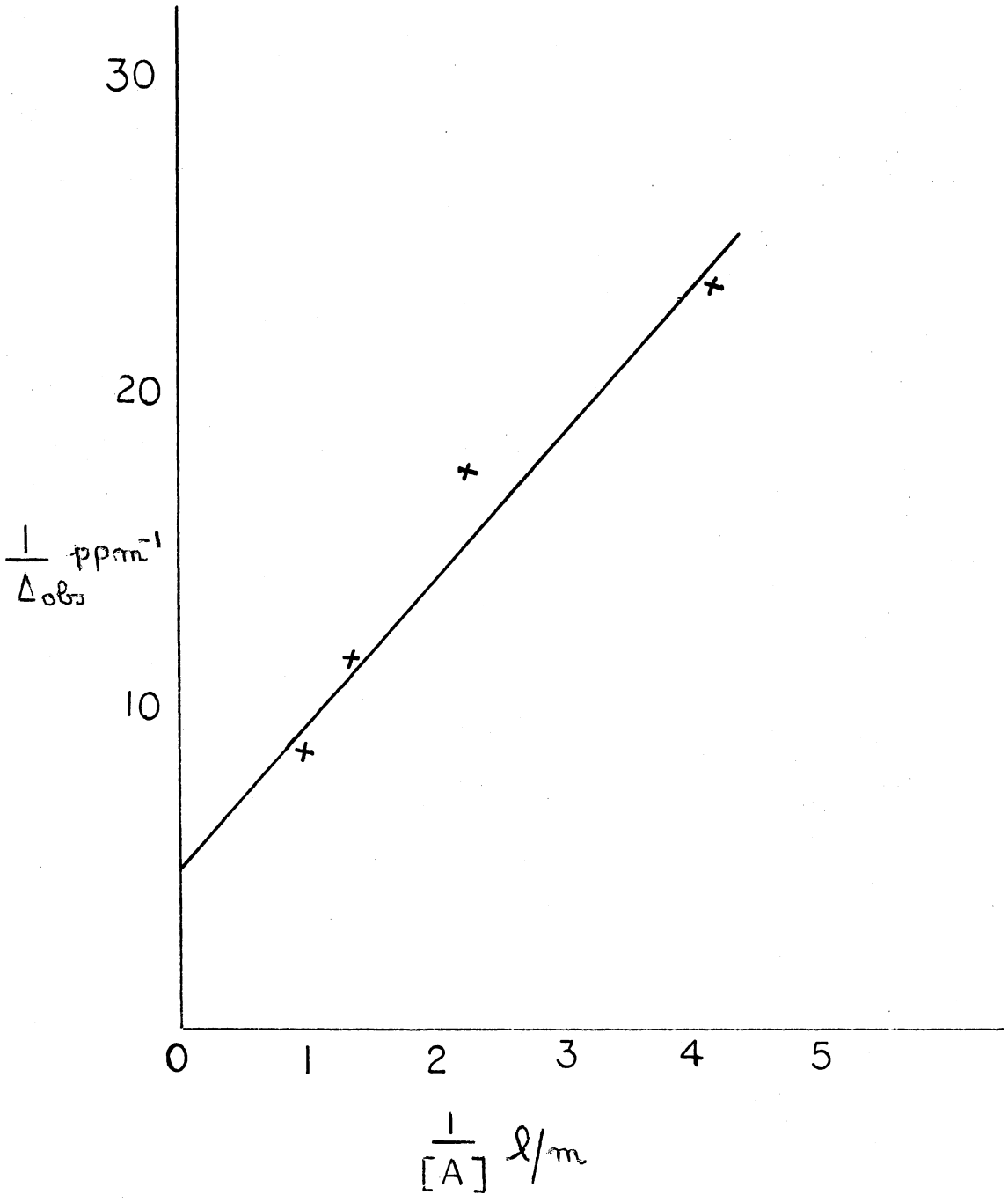


Figure 11. A Plot of $\frac{1}{\Delta_{obs}}$ ppm^{-1} vs $\frac{1}{[A]}$ ℓ/m for Methylene Protons of the Pyrazole 65 in the Complex

An association constant,[21,23,24,136] K, was calculated for the complex for which the concentration of the donor (pyrazole 65) was kept constant while the concentration of the acceptor (trinitrobenzene) was varied. Methylene protons ($S-CH_2$) were monitored via PMR analysis. The singlet absorption signal and clear pattern of shift trend of the acceptor molecule proved instructive. The results were tabulated in Table XVI.

The equation[21,23,23,117,136] used was:

$$\Delta_{obs} = \frac{[A]K}{1 + [A]K} \Delta_o$$

where $\Delta_{obs} = \delta D_{obs} - \delta D_o$ and $\Delta_o = \delta D_{AD} - \delta D_o$. δD_{obs} = observed shift of the donor protons in the complexing medium; δD_o is the shift of donor protons in the uncomplexed state; and δD_{AD} is the shift of donor protons in the pure complex.

The reciprocal of the equation may be written as:

$$\frac{1}{\Delta_{obs}} = \frac{1}{K\Delta_o} \frac{1}{[A]} + \frac{1}{\Delta_o} .$$

K can be calculated from the Y intercept and slope.

$$K = \frac{\text{Y intercept}}{\text{slope}} = 1/\Delta_o \Big/ 1/K\Delta_o$$

Experimental values of $1/\Delta_{obs}$ ppm^{-1} and 1/[A] ℓ/m were subjected to linear regression of $1/\Delta_{obs}$ ppm^{-1} on 1/[A] ℓ/m and the following values for the slope and Y intercept were obtained.

TABLE XVI

CHEMICAL SHIFT OF METHYLENE PROTONS ACCORDING TO VARIED CONCENTRATIONS OF THE ACCEPTOR

Ratio of Donor to Acceptor	Δ_{obs} in Hz	$\frac{1}{\Delta_{obs}}$ ppm^{-1}	[A] in m/ℓ	$\frac{1}{[A]}$ in ℓ/m
D:A				
1:1	4.3	23.3	0.241	4.1
1:2	5.5	18.2	0.482	2.1
1:3	9.0	11.1	0.723	1.4
1:4	12.0	8.3	0.964	1.0

Slope = 4.68453

Intercept = 5.15325

$$K = \frac{\text{Y intercept}}{\text{slope}} = \frac{5.15325}{4.68453}$$

K = 1.10 ℓ/m

The K value was in fair agreement with the reported K values for similar systems such as the N,N-dimethylaniline-1,4-dinitrobenzene complex[114,115,116,117] (K = 0.05 ℓ/m) and the pentamethylbenzene-1,3,5-trinitrobenzene complex[114,115,116,117] (K = 1.93 ℓ/m).

In our investigation of the molecular complexing formation of another model system between 1,4-dihydro[1]benzothiopyrano[4,3-c]-pyrazole (65) and 4-fluorophenylacetonitrile (122) (4FPAN), a moderate diamagnetic shift in the PMR spectrum was experienced only by CH_2 protons of 4-fluorophenylacetonitrile as shown in Figure 12. A summary of chemical shift differences was given in Table XVII.

As indicated, the shift differences were not as significant as those of the TP-TNB system. The proton signal for CH of the pyrazole was almost unchanged. However, the aromatic ring proton H_a experienced a slight paramagnetic effect (1 Hz).

Evidence for the existence of a complex in this case was obtained by UV analysis, in which the complete disappearance of an R band at 312 nm which is probably due to a $n \rightarrow \pi^*$ transition[232,239] and is shown in Table XVIII. The nonbonding electrons present in the uncomplexed state could be involved in the formation of the complex, perhaps via a charge transfer mechanism. Based on the results obtained, a reasonable structure is given in Figure 13.

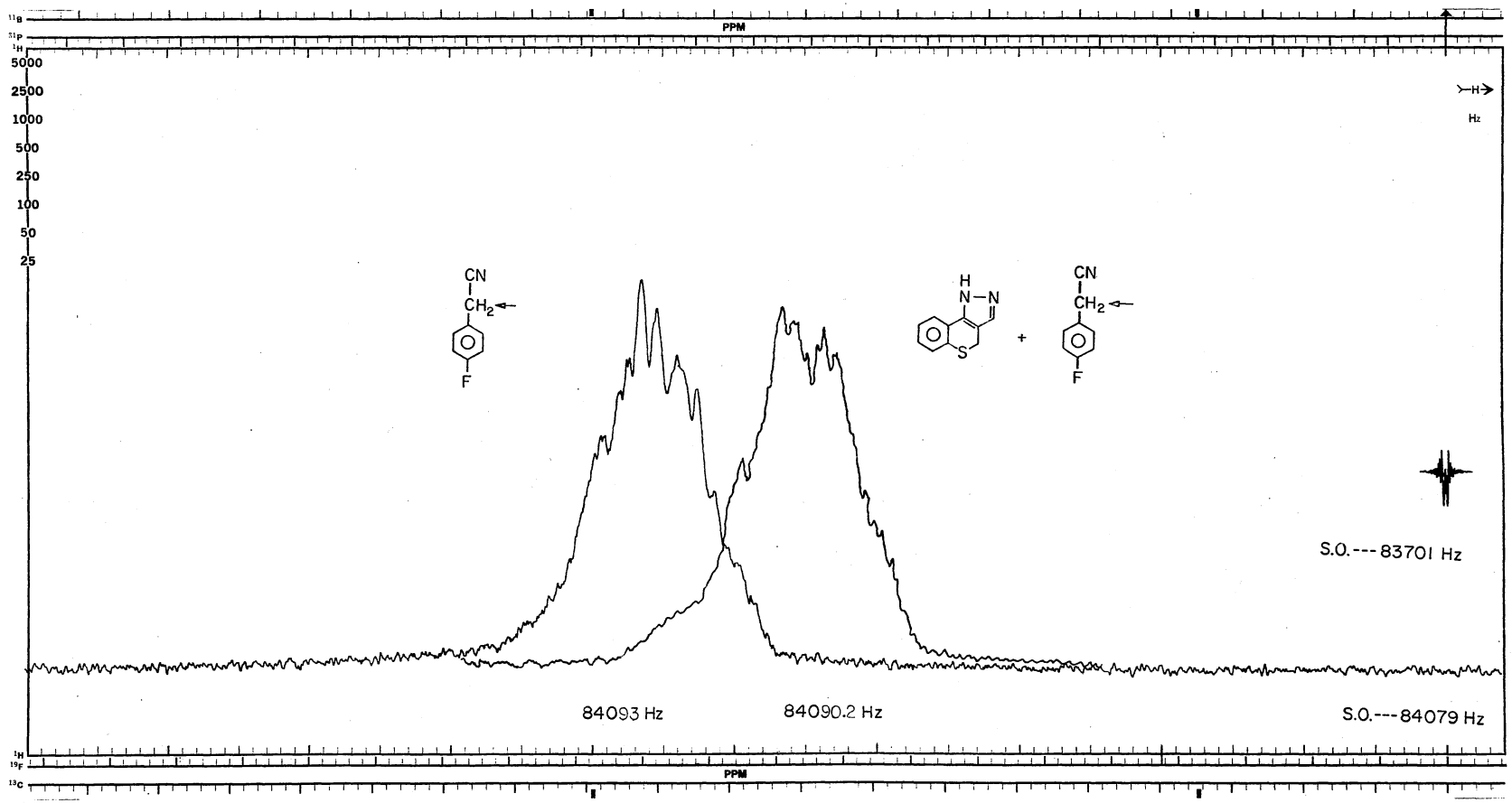


Figure 12. PMR Spectra Showing Chemical Shifts of Methylene Protons of the Nitrile 122

TABLE XVII

CHEMICAL SHIFT CHANGES IN 1:1 COMPLEX OF 1,4-DIHYDRO[1]BENZOTHIOPYRANO[4,3-c]-PYRAZOLE AND 4-FLUOROPHENYLACETONITRILE IN ACETONE-d_6

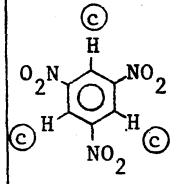
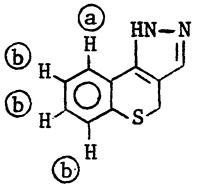
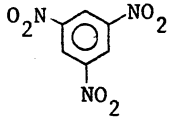
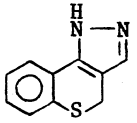
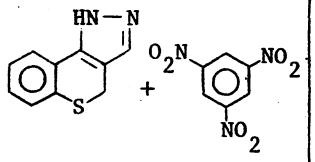
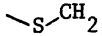
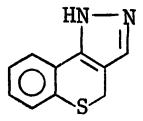
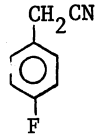
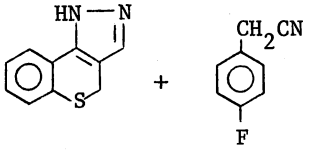
Kinds of Proton with Respect to		Chemical Shifts in H_z			Difference in Chemical Shifts in H_z
		 uncomplexed	 uncomplexed	 complexed	
	CH		752	747	5.0
			400.5	396.2	4.3
	Aromatic (a)		786	778.4	8.4
	Aromatic (b)		723	718	5.0
Aromatic (c)		932.8		925	7.8

TABLE XVIII

ULTRAVIOLET ABSORPTION DATA FOR 1,4-DIHYDRO[1]BENZOTHIOPYRANO[4,3-c]-PYRAZOLE, 4-FLUOROPHENYLACETONITRILE AND THEIR COMPLEX IN 95% ETHANOL

Compounds	Complex	U.V. Absorption	
		Wavelength in nM	ε_{max}
 (9.636×10^{-5} m/l)		312 245	2700 18580
 (1.9272×10^{-4} m/l)		275 270	830 882
	 1:2 Complex (2.8908×10^{-4} m/l)	270 245	1972 5466

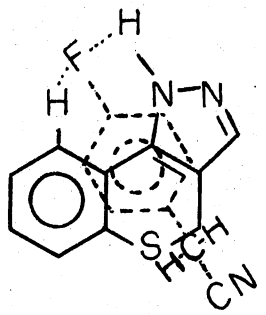
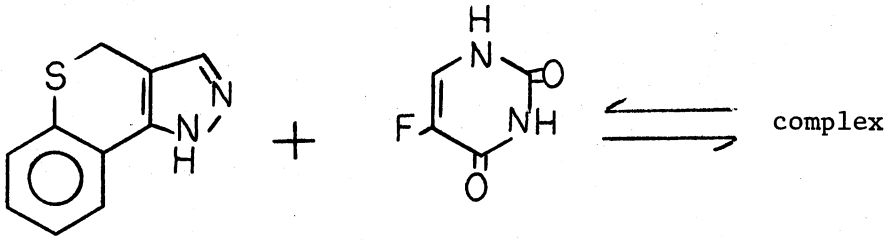


Figure 13. Schematic Representation of TP-4FPAN Complex

Complexation studies involving pyrazole 65 and 5-fluorouracil (42) could not be successfully carried out because of the low solubility of 65 in H_2O or DMSO-d_6. But UV studies were performed at concentrations of 4.6×10^{-5} mole liter per solution of individual compound. The significant observation in the spectra was the hyperchromic effect of E-bands (200 nm - 220 nm), when the mixture was blanked with the pyrazole 65. This may be ascribed to change in electronic transitions in the benzenoid system.[232] Besides this observation, a slight red shift of E-bands was noticed, reminiscent of a π-π^* transition.[232] Although an appreciable interaction between the 5-fluorouracil and the benzene part of the pyrazole 65 molecule was suspected, the arrangement of the donor and acceptor in the complex cannot be discerned at the moment.



65 42

The formation of a molecular complex between 4H[1]benzothiopyrano-[3,4-d]isoxazole (68) and 5-fluorouracil (42) was examined via PMR spectral analysis at a 1:1 molar ratio of solutes (3.17×10^{-4} mole in 0.5 mole of DMSO-d_6). It was conceived that rapid tautomerization[C-12] normally exhibited by 5-fluorouracil might be prevented by interaction with isoxazole 68. Two broad singlets (δ 10.7 and δ 11.5) appeared in the NMR spectrum when the isoxazole and 5-fluorouracil were mixed (Figure 14). These two peaks were absent in the individual species and might arise from possible hydrogen-bond formation as shown in Scheme 23. These data must be treated as tentative in view of the known dependence for field position of acidic protons on the degree of acidity.

It was also found in UV spectral analysis that the B-bands underwent a slight bathochromic shift, which might presumably have resulted from a reduction in the energy level of the excited state accompanying dipole-dipole interaction and hydrogen bonding.[232] This supports the proposed development of H-bonding, and, as shown in Scheme 23, a preferred organization of both the donor and acceptor in solution is quite reasonable.

Complexation studies between 4,5-dihydro-6,7,8-trimethoxy-1H-benz-[g]indazole (132) and 5-fluorouracil (42) were also undertaken (3.17×10^{-4} mole in 0.5 ml of DMSO-d_6) via PMR spectral analysis. To improve solubility, 0.5 ml more of DMSO-d_6 was added to the mixture (for a total volume of 1 ml). The PMR spectral analysis revealed two interesting phenomena. A doublet at δ 7.72 (= C-H) of 5-fluorouracil was transformed into a broad signal which appeared at δ 7.66-7.88 in the 1:1 mixture. When the concentration of the acceptor (3.17×10^{-4} mole) was doubled, the broad signal sharpened. The singlet aromatic signal

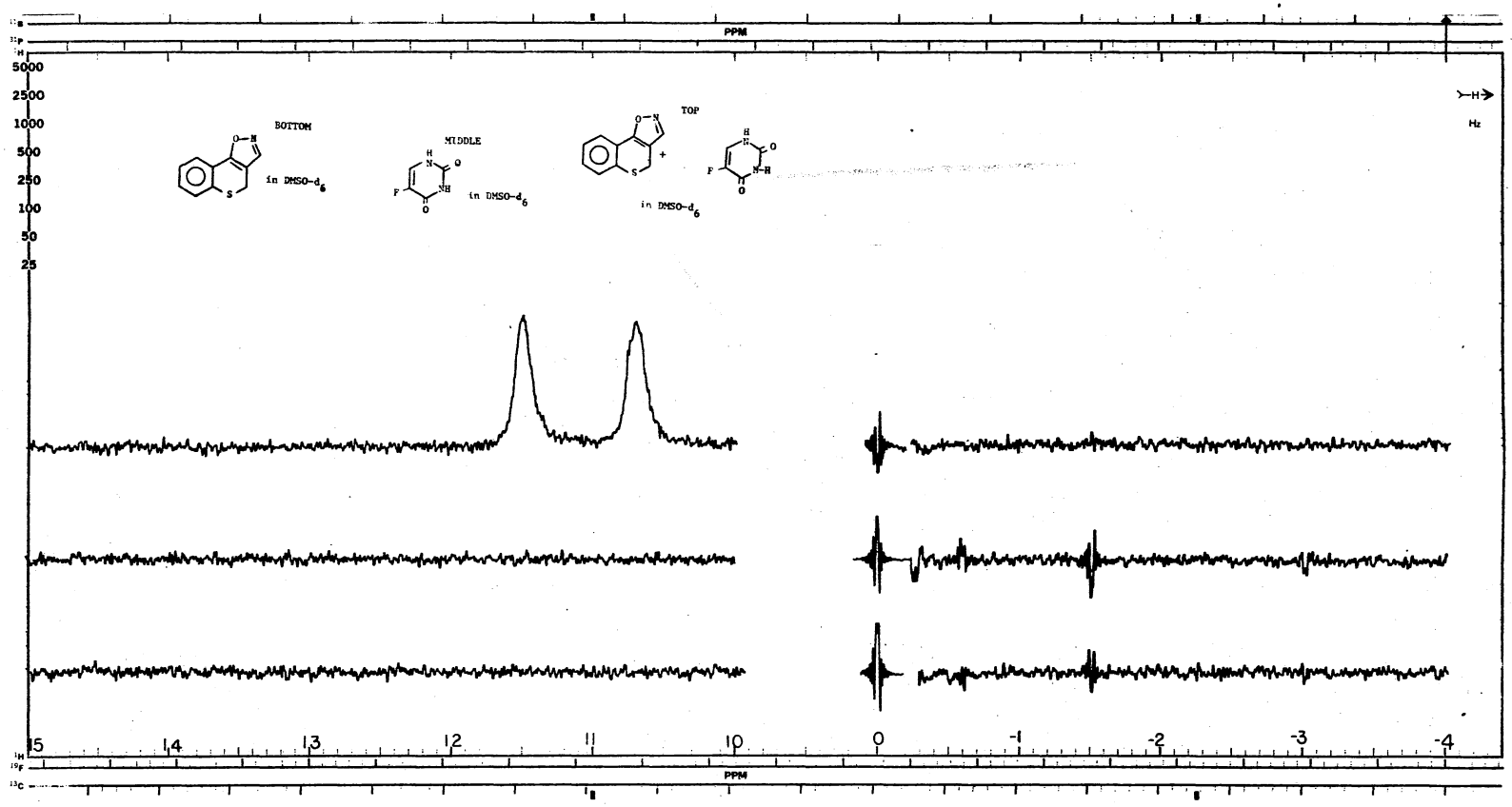
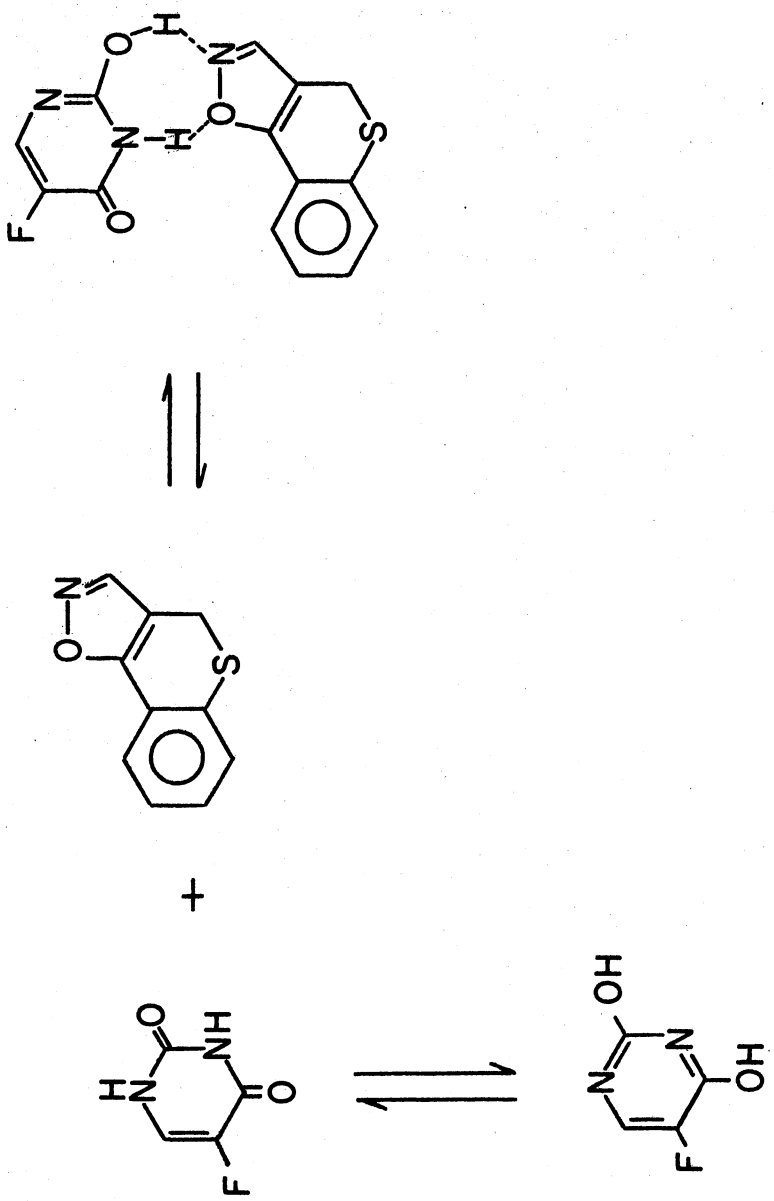


Figure 14. PMR Signal Observed for Acidic Protons in Isoxazole 68-5-Fluorouracil Complex System



Scheme 22

exhibited a diamagnetic shift of 0.023 ppm for the 1:1 mixture and 0.033 ppm upfield for the 1:2 mixture. These values were obtained via analysis of results in scale-expanded regions from δ 7-8.

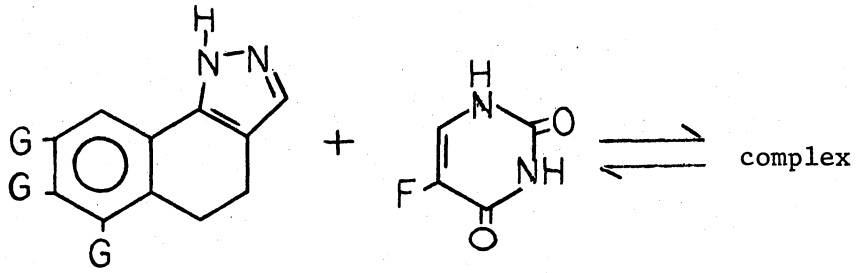
As a crude approximation, the shift differences substituted into the previously cited equation gave a K value of 2.43 ℓ/m. Though the

$$\Delta_{obs} = \frac{[A]K}{1 + [A]K}\,\Delta_o$$

$$0.01 = \frac{3.17 \times 10^{-1}K}{1 + 3.17 \times 10^{-1}K}\,0.023$$

$$K = 2.43\ \ell/m$$

K value seems reasonable, we cannot accept it with great confidence because of an insufficiency of 132, for which 3-4 more values were needed for Δ_{obs} and Δ_o. However, there is no doubt that the donor and acceptor interact with each other. Since signals due to the aromatic proton and FC=CH proton were perturbed in the mixture, a π-σ type complex is suspected. With the data available the spatial arrangement



G=OCH_3 132 42

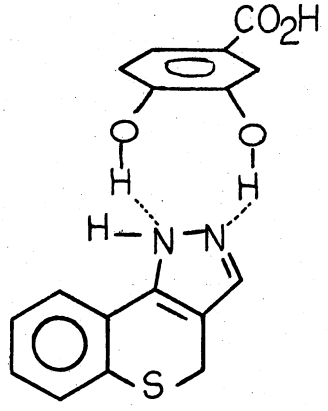
of both the donor and acceptor atoms cannot be described with absolute accuracy.

Although 3,4-dihydroxybenzoic acid (123) was not a good candidate for molecular complexation studies (because it possesses both donor as

well as acceptor characteristics) it was selected for complexation studies with pyrazole 65 in view of the recent report by Durham and Keudell.[86a] They observed that the inhibition of synthesis of amidase by actinomycin D, a clinical anticancer agent, could be prevented or alleviated by 3,4-dihydroxybenzoic acid and suggested that the two compounds formed a complex that (in this case) inactivated the antibiotic.

Equimolar quantities of "free" molecules (2.409×10^{-4} mole each in 1 ml of acetone-d_6) were employed. Line broadening was observed for the hydroxyl protons; the low spin-spin relaxation (small T_2 values) may be due to self-interaction of 3,4-dihydroxybenzoic acid in solution. Other possibilities[53a] for line broadening are magnetic inhomogeneity or an increase in bulk viscosity as well as self association and/or intermolecular complex formation. Since our investigation was carried out in dilute aqueous solution, the viscosity variable is unlikely.

When pyrazole 65 was added, larger T_2 values were noted; this may be due to suppression of self-interaction of 3,4-dihydroxybenzoic acid by the pyrazole moiety. Although it is not illogical to conclude that formation of an intermolecular complex has occurred as shown, the dependence of δ values of acid protons[53a] on concentration precludes elimination of alternative structures and explanations.



Spin-spin relaxation time (T_2 values) is an important technique to study the motion of a part of a molecule in NMR spectroscopy.[53a] When nuclei are sufficiently close to each other, a realignment of atoms can occur. In such compounds, nuclei may exchange spin states, and the process of mutual reorientation is hence spoken of as spin exchange. This relaxation time, T_2, is related to the width of an absorption line at half intensity.[53a] If the shape of the resonance line is given by a Lorentzian curve, T_2 is given by the reciprocal of the half-width at half-height. Thus any decrease of T_2 will manifest itself as a broadening of the absorption line.[53a]

The temperature dependence of spin-spin relaxation time was examined for thiochroman pyrazole-3,4-dihydroxybenzoic acid and the data are tabulated in Table XIX. As the temperature increased, the relaxation time began to increase; dissociation of the complex was suspected.[53a]

Other data supporting the presence of a complex were found via UV spectral analysis (4.6×10^{-5} molar solution in ethanol was used). When blanked with 3,4-dihydroxybenzoic acid, the spectrum exhibited a new absorption peak at 225 nm ($\varepsilon_{225} = 7609$) with a concomitant bathochromic shift of the signal at 245 nm to 250 nm.

Biological Activity

In cooperation with a group in the Microbiology Department, headed by Professor N. N. Durham, it was possible to evaluate the activity of compounds on the growth of microorganisms and KB cells. The primary screening was performed to study growth alteration of Bacillus subtilis by 91 μg/ml of test compound. This screening process was carried out

with Pseudomonas fluorescens and KB cells before screening in mice.

TABLE XIX

TEMPERATURE DEPENDENCE OF THE SPIN-SPIN RELAXATION TIMES OF 3,4-DIHYDROXYBENZOIC ACID-1,4-DIHYDRO[1]-BENZOTHIOPYRANO[4,3-c]PYRAZOLE MIXTURE

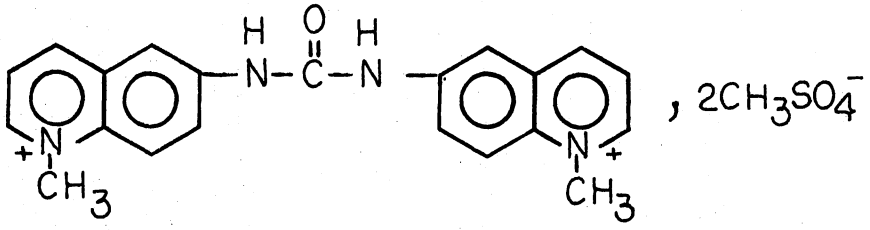
Temperature in °C	T_2 in Seconds
0	1.8181
10	2.1052
20	2.6667
30	2.8571
40	3.3333

It is interesting to note that thiochromanopyrazole 65 has been found to inhibit growth in B. subtilis even at a concentration of 75 µg/ml. This also exhibited 72% plating efficiency on 12.5 µg/ml and a substantial effect was observed at 50 µg/ml (0% plating efficiency, 100% inhibition). In contrast, the corresponding sulfone 70 showed no growth inhibition of B. subtilis or Ps. fluorescens. But, the respiration test (with B. subtilis) with 70 showed an inhibition as was the case with pyrazole 65. Surprisingly, the sulfone derived from thiochroman-4-one (73) produced a 4-hr lag in growth (B. subtilis) along with positive results for respiration screen (B. subtilis, Ps.

fluorescens, SA-180, L-1210). Isoxazole 68 inhibited growth of B. subtilis (overnight) and KB cells (at 50 μg/ml, 100% inhibition of cell growth resulted); but Ps. fluorescens was unaffected. The corresponding sulfone 72 showed no apparent biological activity. Biological testing using higher concentrations of these test compounds has yet to be done. Since it has been established that both qualitative and quantitative responses of man and other species to cancer drugs differ considerably, inhibition of KB cell growth alone may not be considered as a criterion for possible drug activity in humans.

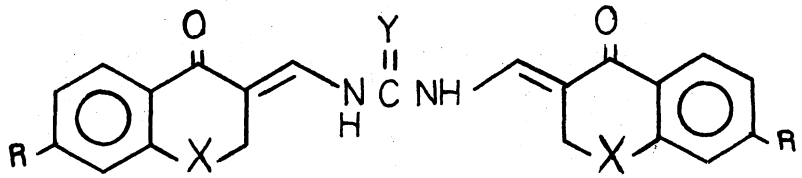
Suggestions for Future Work

It has been reported[41] that the most widely used agent for the treatment of babesiasis at present is quinuronium methosulfate (135).



135

Preparation of 136, 137, 138, and 139 seems worthy owing to their structural similarity to the compound 135. Starting materials are available (Aldrich Chemical Company, Inc.) and a reasonable procedure for the synthesis of 136, 137, 138, and 139 is available.



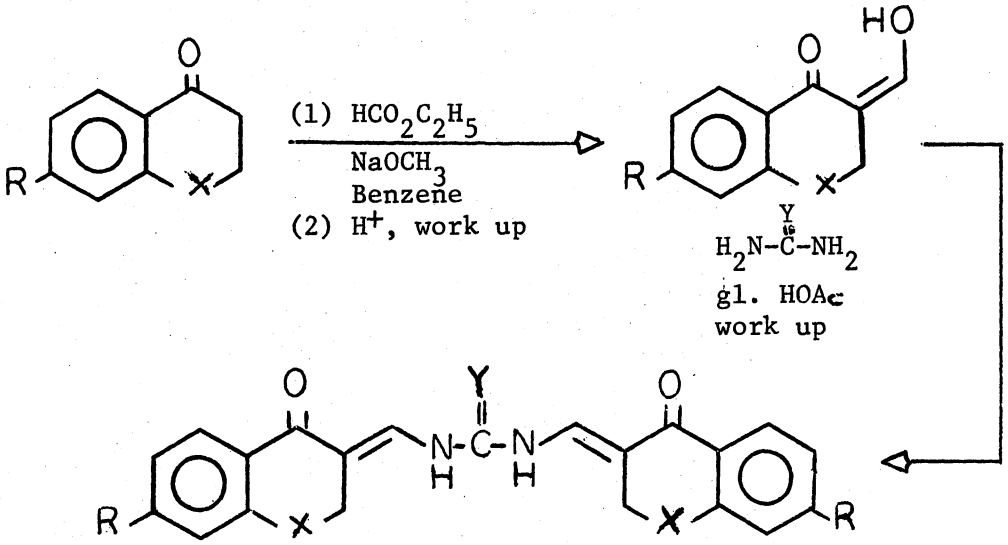
136 X = CH_2; Y = O; R = OCH_3

137 X = CH_2; Y = S; R = OCH_3

138 X = S; Y = O; R = H

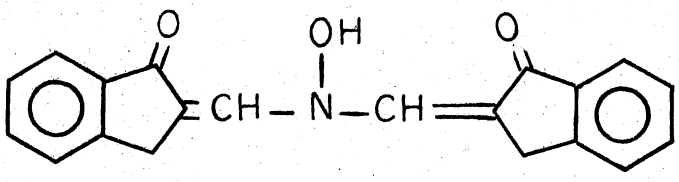
139 X = S; Y = O; R = H

The following synthetic scheme is proposed.



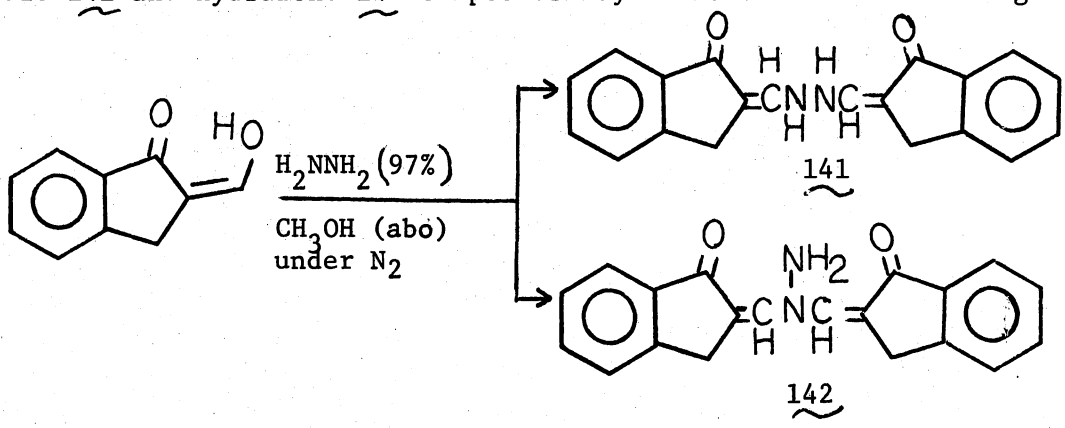
The existence of various possible tautomeric forms might create great difficulties for unequivocal determination of these structures. Aqueous solubility can be improved by converting 138 and 139 to their salts.

Johnson and Shelberg[141] have prepared the bis indanone derivative 140 (although the proposed structure is questionable since very little



140

evidence was furnished in support of it) and explained the formation on the basis that strain prevents cyclization to isoxazole derivatives. By applying the same argument (but different reagents), the following hydrazo 141 and hydrazono 142 compounds may be made on the following

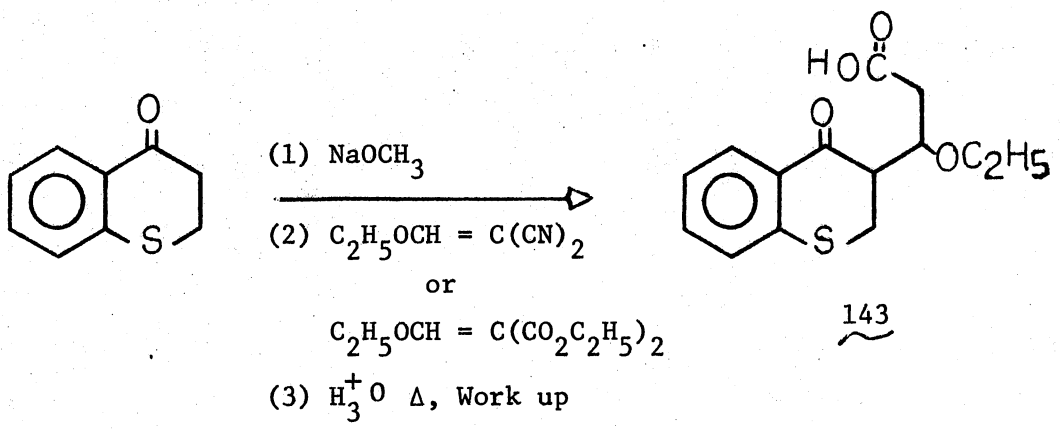


141

142

tentative (but plausible) scheme. The structure determination may be achieved by condensation of the hydroxymethylene compound with various substituted hydrazines. 1,2 Substituted hydrazines cannot undergo the reaction yielding compounds of type 142. Pyrazole formation can also occur (contrary to Johnson and Shelberg 141).

Preparation of a propionic acid derivative 143 of thiochroman-4-one may be accomplished by the following route. Once the preparation of 143 is successfully completed, equilinin-type model compounds may readily be prepared by procedures perfected by Dr. Berlin's research group, Department of Chemistry, Oklahoma State University, Stillwater, Oklahoma.

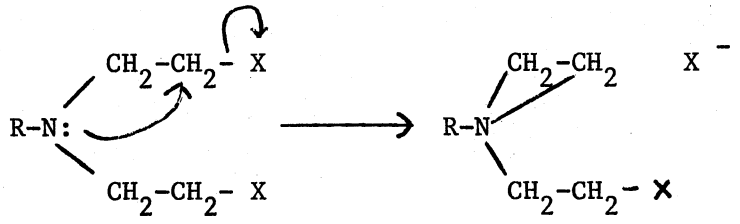


A long-known drug, sulfonal, has the following structure. Com-

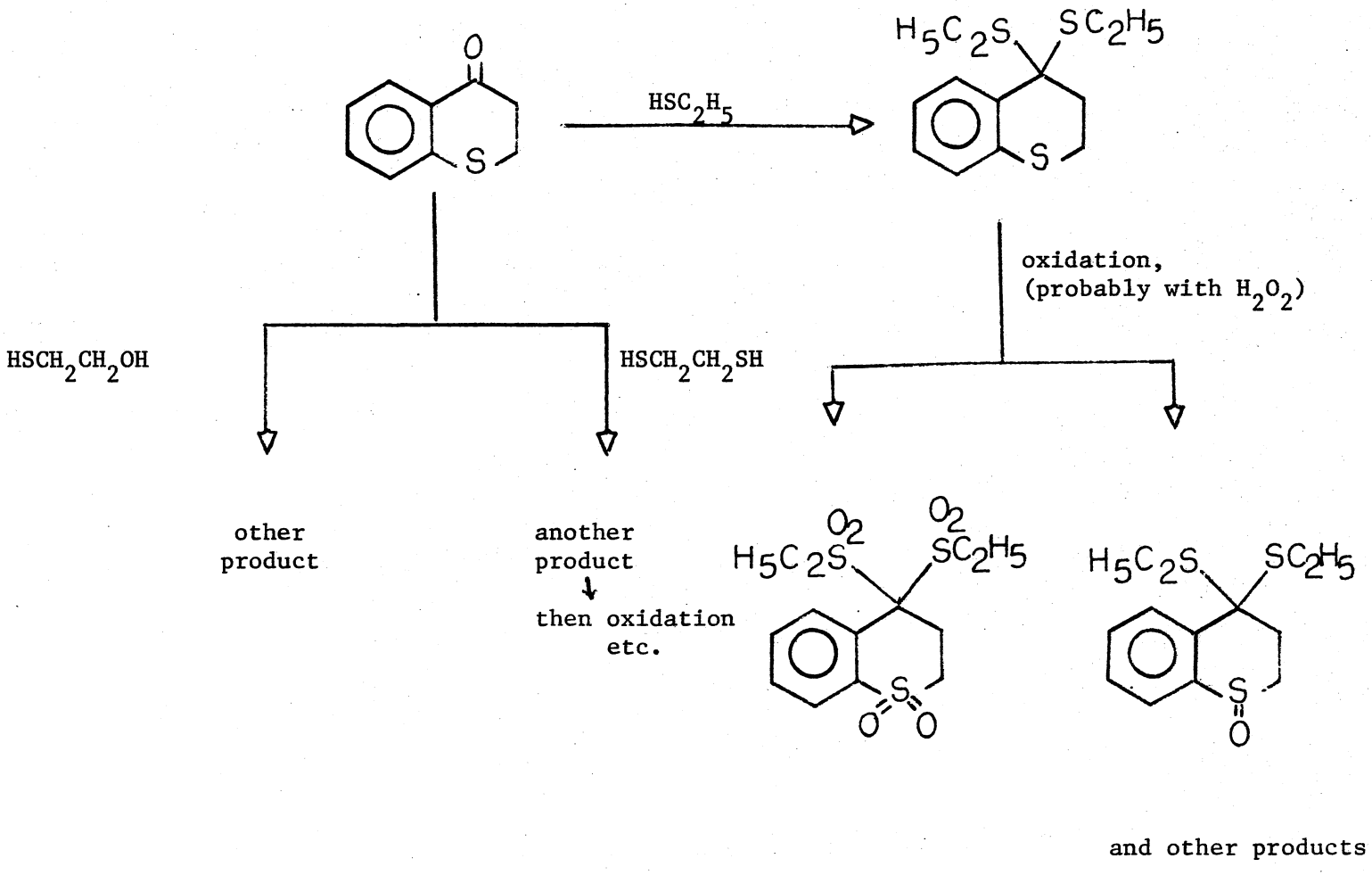
$$(CH_3)_2C(SO_2C_2H_5)_2$$

pounds having similar functionalities can be prepared from readily available materials as shown on page 136.

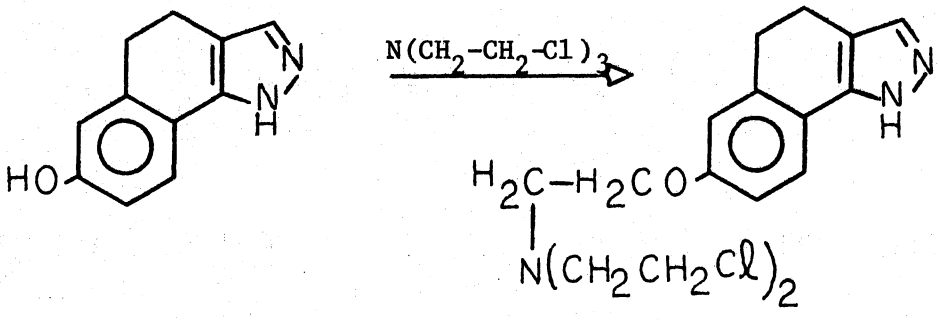
A common mechanism of action in one type of drugs used in cancer chemotherapy (alkylating agent) appears to be the one given below.



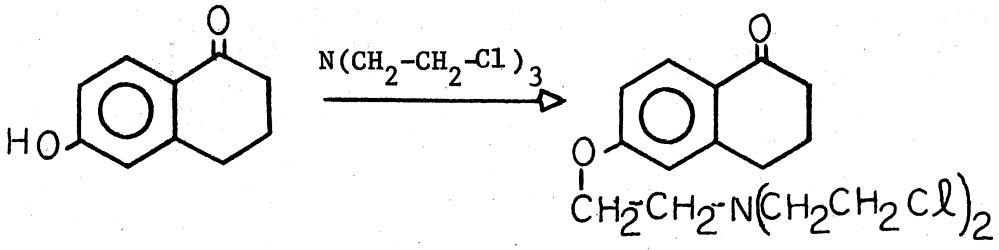
So similar functionaliities may be worth introducing into some of the systems such as those shown on page 137. Though the procedure seems

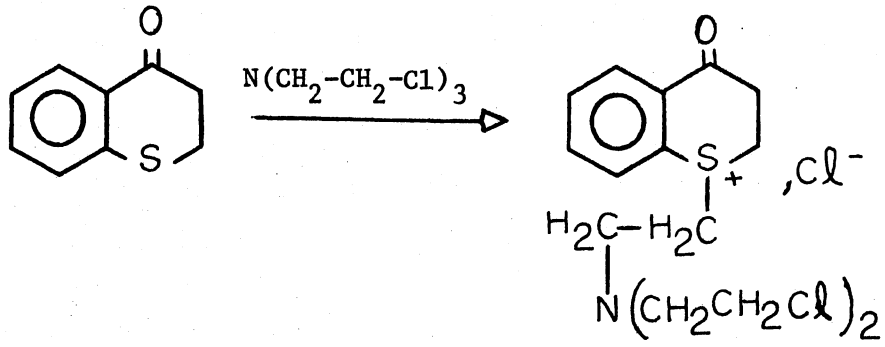

O
S
HSC_2H_5
H_5C_2S
SC_2H_5
S
oxidation,
(probably with H_2O_2)
$HSCH_2CH_2OH$
$HSCH_2CH_2SH$
other
product
another
product
then oxidation
etc.
O_2
O_2
H_5C_2S
SC_2H_5
S
O=O
H_5C_2S
SC_2H_5
S=O
and other products

speculative, it is quite plausible (alkylation at NH may be expected too).

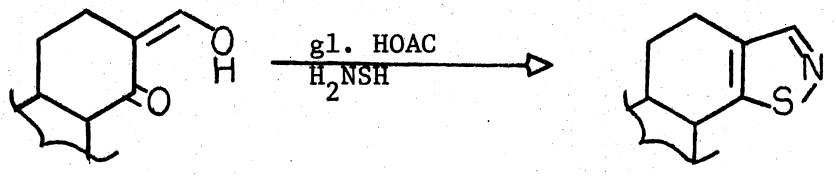


It is even worth trying to make the following compounds, since starting materials are readily available.

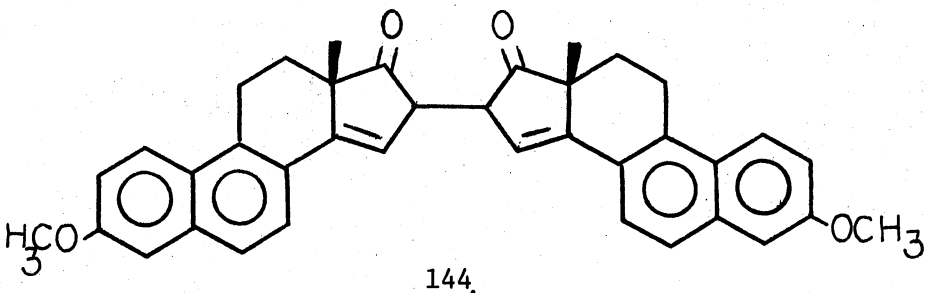




By methods analogous to the formation of isoxazoles, the following compounds may be prepared. The procedure is tentative, but quite reasonable.

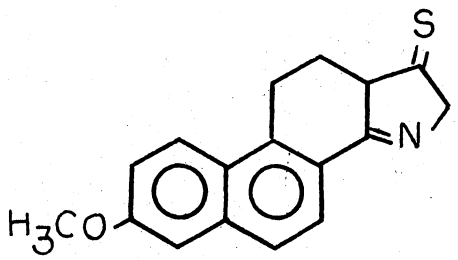


Coupling of two steroidal moieties may be performed by the selection of proper conditions and use of iodine as oxidizing agent (similar reaction agent is known)[207a,224a,243a] to yield the following compound 144.

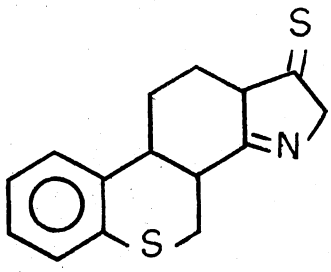


144

The compounds of the types following may also be speculative, but can be obtained by methods developed in our laboratory.



145



146

It is my sincere belief that with better understanding of biochemical interactions, systematic approach to drug design would become more predictive. Complexation studies with compounds having strategically positioned heteroatoms might contribute significantly to this problem. In addition to this, complexation studies between (a) vitamins (vitamin C) and anticancer drugs (e.g., adriamycin, (b) two anticancer drugs, (c) anticancer drugs and cancer-causing agents (carcinogenic compounds) are worthy to be considered. My immediate selection for complexation studies would be adriamycin and digitalis (refer to Chapter I). Adriamycin causes heart disease[252] and it is possible that a combination of this drug and digitalis might not possess this undesirable side effect.

CHAPTER III

EXPERIMENTAL[a-h]

All reactions described herein were performed many times on various scales with slight modifications in procedures. In general, the best results are given and the following are representative descriptions

---

[a]Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected in degrees Centigrade.

[b]Proton magnetic resonance spectra were obtained on a Varian XL-100(15) high resolution NMR spectrometer (with a time-averaging computer accessory, C-1024) operating at 100.1 MHz with tetramethylsilane (TMS) used as an internal reference. The spectra were consistently recorded using a 10 ppm sweep width with the signal from TMS as reference. Any signals which fell beyond 10 ppm were shown with a separate plot of those signals using an offset base-line near the left side of the spectrum. For some compounds, expanded plots were included for key regions for the sake of enhanced resolution. Each spectrum was directly photographed from an original black-ink recording on blue-grid chart with omission of the grid lines.

[c]Carbon-13 NMR spectra were obtained using a 200 ppm sweep width with the signal from TMS appearing at zero using external F-19 lock.

[d]UV spectra were obtained on a Cary 14 Spectrophotometer.

[e]IR spectra were taken on a Beckman-5A spectrophotometer with samples in potassium bromide pellets or films on sodium chloride plates. Each spectrum was directly photographed from an original red-ink recording on golden-grid chart paper.

[f]Low resolution mass spectra were obtained on a CEC 21-100B double focusing mass spectrometer unit. For some compounds peak matchings were carried out using PFK as reference.

[g]Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

[h]Commercially available reagents were used without further purification unless otherwise specified.

of the procedure developed.

Preparation of 2-Hydroxymethylenethiochroman-4-one (94). Commercial sodium methoxide [2.7 g. (0.05 mole); Fisher Scientific Company, "purified" grade] was suspended in 20 ml. of anhydrous reagent-grade benzene in a previously dried 200-ml., 3-necked, r. b. flask fitted with nitrogen inlet. Ethyl formate [3.7 g., 0.05 mole; Matheson Coleman and Bell] was then added and the mixture was cooled to about 10°C with an ice bath and magnetic stirring.

Thiochroman-4-one [Aldrich Chemical Company, 4.1 g. (0.025 mole)] in 25 ml. of anhydrous benzene was added dropwise to the reaction mixture, the temperature being kept at 10-15° with an ice bath. After the addition was completed, the reaction mixture was allowed to warm at room temperature, at which it turned to a semi-solid, reddish mass, and stirring was stopped. The mixture was left overnight.

Hydrolysis of the reaction mixture was effected with 100 ml. of ice-cold distilled water, and the resulting organic layer was washed successively with distilled water and with aqueous 10% NaOH. The combined aqueous extracts were washed (ether, 3 x 25 ml.) and then acidified with dil HCl (pH = 6). A brown-colored liquied formed; this was extracted with ether (5 x 25 ml.), washed (satd. NaCl, 25 ml.), and then dried ($MgSO_4$). Evaporation of the ether gave 4.2 g. (87.5%) of 94 as a crude, waxy red oil, which was used in the following procedures without further purification. Characterization of the structure was done by IR and NMR (Plates XIXb and Ib) analysis. The molecular weight found by mass spectral analysis was 192.

Preparation of 4H-[1]Benzothiopyrano[3,4-d]isoxazole (68). 2-Hydroxymethylenethiochroman-4-one (94) (1.8 g., 0.0094 mole) was

dissolved in 30 ml. of glacial acetic acid in a 100-ml. 2-necked, r.b. flask equipped with an addition funnel and water condenser. Hydroxylamine hydrochloride (1 g., 0.0145 mole) in 5 ml. of distilled water was then added dropwise at room temperature with constant stirring (magnetic stirrer). The reaction mixture was heated to a boil for 0.5 hr. and then cooled to room temperature. After stirring overnight, the mixture was triturated with cold water (75 ml.). A crystalline solid separated and was filtered out by suction, washed several times with distilled water, and air dried. It was then recrystallized (C_2H_5OH) to yield 1.5 g. (84.3%) of 68, m.p. 71-73°. The molecular weight determined by mass spectral analysis was 189.

Anal. Calcd. for $C_{10}H_7NOS$: N, 7.41; S, 16.93

Found: N, 7.18; S, 16.98

IR and NMR spectra (Plates XXa and IIa) support the proposed structure for 68.

Preparation of 1,4-Dihydro[1]benzothiopyrano[4,3-c]pyrazole (65). 2-Hydroxymethylenethiochroman-4-one (94) (2.5 g., 0.0130 mole) was dissolved in 40 ml. of anhydrous methanol in a dry 100-ml., 3-necked r.b. flask fitted with an addition funnel and N_2 inlet. Hydrazine (3 ml., 97%) in 10 ml. of anhydrous methanol was added dropwise. An exothermic reaction ensued with darkening of the already reddish-brown methanol solution. The mixture was then heated to a boil for 15 minutes and stirred at room temperature for 4 hr. (magnetic stirrer). Distilled water (75 ml.) was added to the reaction mixture which was heated to boiling with stirring (0.5 hr.). The reaction mixture was then cooled in ice cold water. The yellow crystals formed were

filtered out under suction and washed several times with distilled water. The air-dried yellow powder weighed 2.3 g. (93.8%) (two crops); m.p. 168.5-170°. IR and NMR spectra (Plates XXIa and XVII) were in agreement with the assigned structure for 65.

Anal. Calcd. for $C_{10}H_8N_2S$: N, 14.89; S, 17.02.

Found: N, 14.95; S, 17.08.

Molecular weight by mass spectral analysis was 188.

Preparation of 1,4-Dihydro-1-phenyl[1]benzothiopyrano[4,3-c]-pyrazole (66). The hydroxymethylene compound 94 (1.8 g., 0.0094 mole) was dissolved in 30 ml. of glacial acetic acid in a 100-ml., r.b. flask fitted with a water condenser. Solid phenylhydrazine (1.2 g., 0.011 mole) was then added to the solution at room temperature, and it was stirred with a magnetic stirrer. The reaction mixture was heated to a boil for 10 minutes and then cooled to room temperature. After stirring 6 hrs. more, the mixture was diluted with water (50 ml.), heated to a boil, and then allowed to cool at room temperature. The crystalline solid that separated was filtered off by suction and washed several times with distilled water. The air-dried product was then recrystallized (dil CH_3CO_2H) to yield 2.2 g. (88.7%, m.p. 169-171°) of pyrazole 66. The molecular weight by mass spectral analysis was 264.

Anal. Calcd. for $C_{16}H_{12}N_2S$: N, 10.61.

Found: N, 10.55.

NMR spectrum (Plate IIIa) confirms the proposed structure for 66.

Preparation of 1,4-Dihydro-1-(p-methoxyphenyl)-[1]benzothiopyrano-[4,3-c]pyrazole (67). Hydroxymethylene compound 94 (1.8 g., 0.0094 mole) was dissolved in 55 ml. of glacial acetic acid in a 200-ml., r.b. flask fitted with a water condenser. p-Methoxyphenylhydrazine (1.8 g.,

0.013 mole) was then added to the solution with constant stirring (magnetic stirrer). The reaction mixture was heterogeneous at room temperature, but upon boiling for 10 minutes, it became homogeneous. After stirring 6 hrs. at room temperature, the mixture was diluted with distilled water (50 ml.) and heated to a boil. It was cooled to room temperature. A tarry substance formed and was dissolved in acetone (25 ml.) and kept for one week. A reddish-yellow crystal mass formed; this was filtered out under suction and washed several times with distilled water. The air-dried, yellow pyrazole 67 weighed 0.8 g. (28.98%, m.p. 145-146°). The molecular weight by mass spectral analysis was 294.

Anal. Calcd. for $C_{17}H_{14}N_2OS$: N, 9.52.

Found: N, 9.45.

NMR spectrum (Plate IVa) was in agreement with the reported structure for 67.

Preparation of 4H-[1]Benzothiopyrano[3,4-d]isoxazole 5,5-dioxide (72). To a solution of 0.2 g. (0.00105 mole) of isoxazole 68 in 5 ml. of glacial acetic acid was added 3 ml. of 30% hydrogen peroxide, and the reaction mixture was allowed to stand at room temperature (30 hrs.). The mixture was diluted with 25 ml. of cold distilled water and cooled (ice bath). A white, crystalline solid separated and was filtered off under suction and washed (6 x 25 ml.) several times with distilled water. The air-dried crude isoxazole 72 was then recrystallized (CH_3CO_2H) to give 0.22 g. of 72 (94.8%, m.p. 170-172°). The molecular weight by mass spectral analysis was 221.

Anal. Calcd. for $C_{10}H_7NO_3S$: N, 6.33, S, 14.48.

Found: N, 6.21, S, 14.38.

IR and NMR spectra (Plates XXb and IIb) confirm the reported structure for 72.

Preparation of 1,4-Dihydro[1]benzothiopyrano[4,3-c]pyrazole 5,5-dioxide (70). Pyrazole 65 (0.2 g., 0.00106 mole) was dissolved in 5 ml. of glacial acetic acid. To this solution was added 3 ml. of 30% hydrogen peroxide, and the reaction mixture was allowed to stand at room temperature (50 hrs.). The mixture was concentrated on a rotary evaporator to a small volume (3 ml.), which was diluted with 25 ml. of cold distilled water (as a precaution the concentrating of the reaction mixture containing hydrogen peroxide was done slowly to avoid any possibility of explosion). A crystalline solid separated and was filtered off under suction. It was washed several times (6 x 25 ml.) with ice-cold distilled water and air-dried. The pyrazole 70 was recrystallized (dil CH_3CO_2H) to yield 0.15 g. (64.4%) 70, m.p. 249-250°. The molecular weight determined by mass spectral analysis was found to be 220.

Anal. Calcd. for $C_{10}H_8N_2O_2S$: N, 12.73.

Found: N, 12.69.

The reported structure of 70 is confirmed by IR and NMR spectral analysis (Plates XXIb and Va).

Preparation of 1,4-Dihydro-1-phenyl[1]benzothiopyrano[4,3-c]-pyrazole-2,5,5-trioxide (71). Pyrazole 66 (0.3 g., 0.00114 mole) was dissolved in 10 ml. of glacial acetic acid, and to this solution was added 7 ml. of 30% hydrogen peroxide. The reaction mixture was kept at room temperature (50 hrs.) without stirring. The mixture was then diluted with 25 ml. of distilled water and cooled in the refrigerator. A recrystalline solid separated and was filtered off under suction.

It was washed several times (6 x 25 ml.) with distilled water. The air-dried pyrazole 71 was recrystallized (dil CH_3CO_2H) to give 0.35 g. (98.8%) 71, m.p. 211-212°. The molecular weight by mass spectral data was 312.

Anal. Calcd. for $C_{16}H_{12}N_2O_3S$: N, 8.97.

Found: N, 9.04.

The proposed structure of 71 was supported by NMR spectral analysis (Plate IIIa).

Preparation of Thiochroman-4-one-1,1-dioxide (73). Thiochroman-4-one (90) (2.05 g., 0.014 mole) was dissolved in 25 ml. of glacial acetic acid and to this solution was added 10 ml. of 30% hydrogen peroxide. The mixture was allowed to stand at room temperature (50 hrs.). The reaction mixture was then concentrated to a small volume (10 ml.) (rotary evaporator) taking extra care to avoid any possibility of explosion. The concentrated mixture was diluted with 25 ml. of cold distilled water. A crystalline solid formed and was separated by filtration under suction. Ketone 73 was then washed several times with distilled water (3 x 25 ml.) and recrystallized (dil CH_3CO_2H) to yield 1.8 g. (65.7%) 73, m.p. 131-133°. The molecular weight determined by mass spectral analysis was 196.

Anal. Calcd. for $C_9H_8O_3S$: S, 16.33.

Found: S, 16.40.

The proposed structure for 73 was confirmed by NMR spectral analysis (Plate Vb).

Preparation of 1-[(4-Oxothiochroman-3-yl)methylene]-2-thiourea (78). The 2-hydroxymethylene compound 94 (2.7 g., 0.014 mole) was dissolved in 20 ml. of glacial acetic acid. A suspension of thiourea

(1.5 g., 0.019 mole) in 20 ml. of glacial acetic acid was added to the solution and the resulting heterogeneous mixture was stirred for 14 hrs. at room temperature using a magnetic stirrer. As the reaction progressed, the medium became homogeneous. It was then heated to a boil for 10 min. and allowed to stand for 14 hr. at room temperature. The reaction mixture was diluted with cold water (75 ml.) for 30 min. Crystalline solid separated and was filtered off under suction, washed several times with cold water (6 x 20 ml.) and air dried. Recrystallization from dil CH_3CO_2H gave 1.1 g. (31.4%) 78, m.p. 184-186°. The molecular weight by mass spectral analysis was 250 (Calcd. for $C_{11}H_{10}N_2OS_2$). Peak matching using PFK was in good agreement with the proposed structure 78.

M.S. Calcd. for $C_{11}H_{10}N_2OS_2$: m/e 250.117349 (M^+)

Found: m/e 250.012960 (M^+)

Calcd. for fragment $C_{10}H_8NOS$: m/e 190.032658

Found: m/e 190.026492

Calcd. for fragment C_9H_6OS: m/e 162.013935

m/e 162.019275

It was further supported by IR and NMR spectral data (Plates XXIIIa and VIa). However, elemental nitrogen analysis did not indicate the sample to be pure.

Anal. Calcd. for $C_{11}H_{10}N_2OS_2$: N, 11.20.

Found: N, 7.45.

Preparation of 2-Hydroxymethylene-6-methoxy-1-tetralone (93). Sodium methoxide (3.72 g., 0.0672 mole) was suspended in 35 ml. of dry benzene, ethyl formate (5.1 g., 0.0672 mole) was then added to the suspension, and the mixture was cooled to 10° (ice-bath). The system

was kept under N_2 and stirred (magnetic stirrer). A solution of 6-methoxy-1-tetralone in 40 ml. of dry benzene was added and the reaction mixture turned blue. When the mixture was warmed to room temperature, it deposited a yellowish-brown precipitate and was allowed to stand overnight at room temperature without stirring.

Hydrolysis of the reaction mixture was effected with 400 ml. of ice-cold water, and the resulting organic layer was washed successively with distilled water (30 ml.) and with aqueous 5% NaOH (20 ml.). The aqueous portions were combined, washed with ether (3 x 30 ml.) and then acidified with dil HCl and ice (pH = 6). A brown crystalline solid formed and was separated by filtration under suction. It was washed several times (6 x 25 ml.) with distilled water and air-dried to produce 6.7 g. (95.7%) of 93, m.p. 66-68°. This solid was used without further purification. The structure of the hydroxymethylene compound 93 was confirmed by NMR analysis (Plate VIIb).

Preparation of [(1,2,3,4-Tetrahydro-6-methoxy-1-oxo-2-naphthyl)-methylene]urea (76). The 2-hydroxymethylene compound 93 (1.8 g., 0.0088 mole) was dissolved in 30 ml. of glacial acetic acid, and urea (1 g., 0.0166 mole) was added to the solution. The resulting reaction mixture was stirred (10 hr.) at room temperature (magnetic stirrer). It was then boiled for 10 min., cooled to room temperature, diluted with cold water (75 ml.), and let stand for 30 min. Crystals formed and were separated by filtration under suction. They were washed several times with cold distilled water (6 x 25 ml.) and air-dried. The product was recrystallized (dil CH_3CO_2H) to be a 1.8 g. (83.2%; m.p. 235-237°) of 76. Molecular weight found by mass spectral analysis was 246.

Anal. Calcd. for $C_{13}H_{14}N_2O_3$: N, 11.38.

Found: N, 11.15.

IR and NMR spectral analysis (Plates XXVIIIa and VIIIa) agreed with the reported structure for 76. This structure was reconfirmed by peak matching using PFK as follows:

M.S. Calcd. for fragment $C_{12}H_{12}NO_2$: m/e 202.086798

Found: m/e 202.092692

Calcd. for fragment $C_{12}H_{13}NO_2$: m/e 203.103174

Found: m/e 203.099577

Calcd. for fragment $C_{12}H_{12}O_2$: m/e 188.083724

Found: m/e 188.070020

Preparation of 1-[(1,2,3,4-Tetrahydro-6-methoxy-1-oxo-2-naphthyl)-methylene]-2-thiourea (77). The hydroxymethylene compound 93 (2.5 g., 0.0123 mole) was dissolved in 40 ml. of glacial acetic acid and thiourea (1.5 g., 0.0197 mole) was added to the solution. The resulting reaction mixture was stirred overnight at room temperature (magnetic stirrer). It was then boiled for 10 min. and tirturated with cold distilled water (50 ml.) for 30 min. A crystalline solid separated and was filtered out under suction. It was then washed (3 x 25 ml.) several times with cold water and air-dried. The product was recrystallized (dil CH_3CO_2H) to yield 3.2 g. (99.3%, m.p. 225-227°) of 77.

Anal. Calcd. for $C_{13}H_{14}N_2O_2S$: N, 10.68; S, 12.21.

Found: n, 10.52; S, 12.33.

IR and NMR spectral analysis (Plates XXIIIb and VIIIb) were in agreement with the proposed structure for 77. The structure was further supported by peak matching using PFK.

M.S. Calcd. for $C_{13}H_{14}N_2O_2S$: m/e 262.077593 (M^+)

Found: m/e 262.076703 (M^+)

Calcd. for fragment $C_{12}H_{12}NO_2$: m/e 202.086798

Found: m/e 202.084764

Preparation of Methyl 6-Methoxy-2-methyl-1-oxo-1,2,3,4-tetrahydro-naphthalene-2-carboxylate (97). A mixture of 6-methoxytetralone (6.82 g., 0.0388 ml.), dimethyl carbonate (41.6 ml.) and sodium methoxide (2.42 g., 0.0448 mole) were boiled under N_2 for 2.5 hr. A yellowish precipitate formed. The mixture was allowed to cool and 200 ml. of absolute methanol was added to dissolve the precipitate. A solution of methyl iodide (9.14 g., 0.0644 mole) in 200 ml. of absolute methanol was added, and the mixture was stirred overnight at room temperature. The mixture turned greenish in color and was then boiled for 10 min. and adjusted with 2N acetic acid to pH 6. Upon cooling, compound 97 crystallized as yellow solid. Recrystallization from methanol gave 54 g. (82%) of 97, m.p. 91-93.5° (recorded m.p. 91-92°) (IR Plate XXIVa).

Preparation of 2,3a,4,5-Tetrahydro-3a-methyl-7-methoxy-3H-benz[g]-indazol-3-one (79). A mixture of keto ester 97 (14.8 g., 0.059 mole) and 95% hydrazine (1.92 g., 0.060 mole) was stirred at ambient temperature under N_2 for 4.0 hr. As the reaction mixture thickened, absolute methanol (50 ml.) and additional 50% hydrazine (5.0 g.) were added to keep the mixture fluid. At the end of the reaction period, distilled water (200 ml.) was added and the mixture was stirred (45 min.). The product was filtered out and washed (3 x 100 ml. of water) to give 12.29 g. (90.5%) 79, m.p. 217.5-218.5°. An analytical sample of the indazol-3-one purified by sublimation (178°, 0.1 mm., m.p., 218-219°)

gave the following analysis.

Anal. Calcd. for $C_{13}H_{14}N_2O_2$: C, 67.81, H, 6.12; N, 12.17.

Found: C, 67.69, H, 6.22; N, 12.34.

IR and NMR analysis support the proposed structure for 79 (IR Plate XXIVb).

Preparation of 2,10,11,11a-Tetrahydro-7-methoxy-11a-methyl-1H-phenanthro[1,2-c]pyrazol-one (83). 3,4-Dihydro-7-methoxy-1(2H)-phenanthrone (101) (1.75 g., 0.0078 mole) in 45 ml. of anhydrous dimethyl carbonate was stirred (magnetic stirrer) for 15 min. at room temperature under N_2. Sodium methoxide (0.484 g., 0.0098 mole) was then added to the reaction mixture which was heated to a boil. The mixture turned dark-red and gradually deposited a yellow precipitate. Heating was stopped after 75 min.; stirring was continued for 15 min. more. When the reaction mixture reached room temperature, 30 ml. of absolute methanol was added to dissolve the dark-red precipitate. A solution of methyl iodide (0.852 g., 0.006 mole) was then added to the reaction mixture and this was stirred overnight at room temperature. Excess methyl iodide (1 ml.) was again added, and the solution was heated gently for 5 min., cooled to room temperature, and then acidified with 2N acetic acid (pH = 6). The mixture was concentrated to a small volume (50 ml.) on a rotary evaporator.

The resulting tarry substance was triturated with cold distilled water (75 ml.). The viscous layer was extracted with ether (2 x 35 ml.) and washed with distilled water (3 x 35 ml.) and then with saturated NaCl (30 ml.) and finally dried ($MgSO_4$).

Evaporation of the ether gave a viscous liquid which was dissolved in 30 ml. of absolute methanol. Hydrazine (6 g., 95% was added to the

reaction mixture under N_2. A yellowish precipitate formed after 3 hr. of continuous stirring (magnetic stirrer). After 2 hr., the mixture was diluted with 75 ml. of distilled water for 30 min. The product was filtered off under suction, washed (6 x 30 ml.) several times with distilled water, and air-dried to give 1.54 g. (70.6%) of 83, m.p. 232-236°. It was purified by sublimation (150°, 0.01 mm.) to give an analytical sample (m.p. 258-260°). The molecular weight determined by mass spectral analysis corresponds to 280.

Anal. Calcd. for $C_{17}H_{16}N_2O_2$: C, 72.86; H, 5.71; N, 10.00.

Found: C, 72.73; H, 5.62; N, 9.86.

IR and NMR analysis (Plates XXIX and XIb) agree with the proposed structure for 83.

Preparation of 4,5-Dihydro-7-methoxynaphth[2,1-d]isoxazole (87). [This compound was prepared by four different variations of a method in order to determine whether two isomers formed and to study the structures via PMR and ^{13}CMR analysis.] Method A. 2-Hydroxymethylene compound 93 (1.38 g., 0.0075 mole) was dissolved in 30 ml. of glacial acetic acid and hydroxylamine hydrochloride (0.59 g., 0.0085 mole) in 5 ml. of distilled water, was then added to the reaction mixture at room temperature with constant stirring (magnetic stirrer). The mixture was stirred for 48 hr. A red precipitate formed, was filtered out under suction, washed several times (6 x 25 ml.) with distilled water, and air-dried to give 1.2 g (88%) of 87, m.p. 59-61°. Recrystallization of this solid from C_2H_5OH gave a crystalline material, m.p. 59-61°. The proposed molecular weight (201) was confirmed by mass spectral analysis.

Anal. Calcd. for $C_{12}H_{11}NO_2$: C, 71.64; H, 5.47; N, 6.97.

Found: C, 72.01; H, 5.47; N, 6.92.

PMR and ^{13}CMR spectra (Plates IXa and Xa) support the suggested isomer of the compound 87.

Method B. 2-Hydroxymethylene compound 93 (1.72 g., 0.0084 mole) was dissolved in 25 ml. of acetic acid. A solution of hydroxylamine hydrochloride (0.86 g., 0.0124 mole) and sodium acetate (0.86 g., 0.0105 mole) in 5 ml. of distilled water was added to the mixture, and it was then boiled for 1 hr. and cooled to room temperature. It was stirred using a magnetic stirrer. Water (5 ml.) was added to promote the formation of precipitate, which was filtered off by suction and washed several times (6 x 25 ml.) with distilled water and finally air-dried to weigh 1.6 g. (95.2%, of 87a, m.p. 59-61°). A mixed melting point determination with 87 confirmed the identity of 87 and 87a. This was further proved by NMR spectral analysis.

Method C. Compound 93 (1.02 g., 0.005 mole) was dissolved in 30 ml. of methanol. A solution of hydroxylamine hydrochloride (1.05 g., 0.015 mole) and sodium acetate (1.5 g., 0.018 mole) in 5 ml. of distilled water was added to the reaction mixture, which was heated gently for 0.5 hr. over a steam bath. Upon cooling to room temperature, the mixture deposited some solid which was filtered out and washed several times (6 x 25 ml.) with distilled water. It was then air-dried to give 0.2 g. of a solid (m.p. 141-143°) which was not further characterized.

The filtrate was concentrated to a small volume ($\simeq$15 ml.), and the resulting waxy liquid was diluted with dilute C_2H_5OH (25 ml.). A greenish gummy substance resulted (0.7 g., 69.6%), which proved by its

NMR spectrum to be identical with 87 and 87a. This, on recrystallization (95% ethanol), gave 0.7 g. (69%) of 87b, m.p. 59-61°.

Method D. To a solution of hydroxylamine hydrochloride (2.1 g., 0.03 mole) in 4 ml. of distilled water was added compound 93 (2.04 g., 0.01 mole) in 50 ml. of pyridine, and the reaction mixture was boiled for 3 hr. with constant stirring (magnetic stirrer). The solution was evaporated to dryness, the residue dissolved in ethanol (95%), and the alcoholic solution kept overnight, without stirring, in the refrigerator. A greenish gummy substance settled down; the solvent was removed by rotary evaporation to give a solid, 2.0 g. (95%). PMR spectral analysis (Plate IXb) revealed the fact that this compound 87b was not the same as that from method A, B, or C. On the contrary the presence of an isomer of isoxazole was suspected and was confirmed by ^{13}CMR spectrum analysis (Plate Xb).

Preparation of 3,4-Dihydro-1,4-benzothiazepin-5(2H)-one (75). To a solution of 0.5 g. (0.0072 mole) of hydroxylamine hydrochloride in 30 ml. of distilled water was added 2 ml. of an aqueous 10% sodium hydroxide and 0.2 g. (0.0012 mole) of thiochroman-4-one. Just enough 95% ethanol (∿ 1 ml.) was added to the reaction mixture to give a clear solution. It was then warmed on a steam bath for 15 min. and cooled in an ice-water bath. A white precipitate formed, this was filtered off, washed several times (6 x 25 ml.) with distilled water, and air-dried to give 0.2 g. (91.7%) of the oxime 74, m.p. 98-100°. The structure of 74 was confirmed by NMR analysis (Plate XIIa) which was consistent with the reported values for 74. Upon standing for one year in an amber-colored bottle, the crude solid oxime changed from a white crystalline

solid to a red, waxy one. The NMR spectrum indicated the formation of the lactam. This phemomenon was again noticed two days after a freshly prepared sample of the oxime had been dissolved in $DCCl_3$. The spectrum of the sample was found to be identical with that of the lactam 75 described earlier. This Beckmann-type rearrangement was further confirmed by the demonstration of the reaction between the oxime and a couple of drops of conc. HCl when these were kept overnight and again by the NMR spectrum of the product (Plate XIIb).

Anal. Calcd. for C_9H_9NO: S, 17.88.

Found: S, 17.85.

Preparation of 2-Hydroxymethylene-2-methoxybenzosuberone (96). To a stirred suspension of sodium methoxide (1.08 g., 0.02 mole) in 20 ml. of dry benzene was added 1.48 g. (0.02 mole) of ethyl formate. The system was kept under N_2 flow and cooled to 10°C by using an ice bath. To this cooled solution was added 2.0 g. (0.0105 mole) of 2-methoxybenzosuberone in 20 ml. of dry benzene. The reaction mixture turned yellow and a yellowish-red precipitate formed after 5 min. This was allowed to stand overnight at room temperature.

Hydrolysis of the reaction mixture was effected with 200 ml. of ice-cold water, and the resulting organic layer was washed successively with distilled water and an aqueous solution of 10% NaOH. The aqueous portions were combined, washed with ether (50 ml.), and then acidified with dil HCl in ice. A reddish-brown, heavy liquid formed and was extracted with ether (3 x 25 ml.), washed (saturated NaCl, 30 ml.), and then dried ($MgSO_4$). Evaporation of the ether gave 2.2 g. (95.9%) of 96 as a waxy reddish-brown oil which was used in the following procedures without further purification. The structure was characterized by NMR

(Plate XIIIb) analysis. The molecular weight determined by mass spectral analysis was 218.

Preparation of 1,4,5,6-Tetrahydro-8-methoxybenzo[6,7]cyclohepta-[1,2-c]pyrazole (85). The 2-hydroxymethylene compound 96 (1.3 g., 0.0059 mole) was dissolved in 40 ml. of anhydrous methanol and to this solution was added 3 ml. of 97% hydrazine with constant stirring (magnetic stirrer). The reaction was carried out under N_2. The mixture was stirred for 6 hr. at room temperature. It was diluted with 75 ml. of distilled water, boiled for 0.5 hr., and cooled to room temperature. Upon cooling in ice, the solution deposited yellow crystals which were filtered off under suction and air-dried to give 0.8 g. (62.9%) of 85, m.p. 91-96°. Recrystallization from ethanol (95%) gave an analytical sample, m.p. 101-103°. NMR spectral data (Plate XIVa) were in agreement with the proposed structure for 85. The molecular weight by mass spectral analysis was 214 (calcd. for $C_{13}H_{14}N_2O$). Peak matching using PFK confirmed the proposed structure for 85.

M.S. Calcd. for $C_{13}H_{14}N_2O$: m/e 214.112591 (M^+)

Found: m/e 214.110607 (M^+)

Anal. Calcd. for $C_{13}H_{14}N_2O$: N, 13.08.

Found: N, 12.98.

Preparation of 5,6-Dihydro-8-methoxy-4H-benzo[3,4]cyclohepta-[1,2-d]isoxazole (84). To a solution of 2-hydroxymethylene compound 96 (0.5 g., 0.0023 mole) in 35 ml. of glacial acetic acid was added hydroxylamine hydrochloride (0.248 g., 0.0036 mole) in 5 ml. of water; this solution was heated gently for 0.5 hr. with stirring (magnetic stirrer) and then cooled to room temperature. After being stirred

overnight, the mixture was diluted with cold water (75 ml.). The red oily substance formed was separated by extraction with ether. Evaporation of ether on a rotary evaporator gave a dark waxy liquid which on recrystallization (95% ethanol) gave 0.4 g. (81.6%) of 84, m.p. 52-53°.

Anal. Calcd. for $C_{13}H_{13}NO_2$: C, 72.55; H, 6.05; N, 6.51.

Found: C, 72.10; H, 6.15; N, 6.28.

NMR spectral analysis (Plate XIVb) confirmed the suggested structure for 84. This was reconfirmed by peak matching using PFK.

M.S. Calcd. for $C_{13}H_{13}NO_2$: m/e 215.094623 (M^+)

Found: m/e 215.094309 (M^+)

Preparation of 2-Hydroxymethylene-6-methoxy-1-Indanone (95). Sodium methoxide (1.08 g., 0.02 mole) was suspended in 20 ml. of anhydrous benzene under N_2. Ethyl formate (1.48 g., 0.02 mole) was then added, and the mixture was cooled to about 10° using an ice bath and with magnetic stirring. A solution of 6-methoxy-1-indanone (2.0 g., 0.0123 mole) in 20 ml. of anhydrous benzene was added dropwise to the reaction mixture, the temperature being kept between 10-15°C (ice bath). After the addition was complete, the reaction mixture was allowed to warm to room temperature and allowed to stand for 2 hr. Hydrolysis was effected with 100 ml. of cold water, and the resulting organic layer was washed successively with distilled water (30 ml.) and with aqueous 10% NaOH (30 ml.). The combined aqueous extracts were washed (ether 3 x 25 ml.) and then acidified with dil HCl to pH = 6. Yellow crystals formed and were separated by filtration under suction. The crystals were washed several times (distilled water, 3 x 30 ml.) and air-dried to give 2.2 g. (94%) of 95, m.p. 149-150°. The proposed structure was confirmed by molecular weight determination (190) via mass

spectral analysis and NMR analysis (Plate XVb).

In a similar preparation, only the reaction time for synthesis was increased to about 12 hr. The yield of 2-hydroxymethylene compound 95 remained unchanged (94%) (m.p. 151°) in what was otherwise an identical preparation.

Preparation of 2-Hydroxymethylene-5,6-dimethoxy-1-indanone (98). To a stirred suspension of sodium methoxide (1.08 g., 0.02 mole) in 30 ml. of dry benzene under N_2 was added 1.48 g. (0.02 mole) of ethyl formate. 5,6-Dimethoxy-1-indanone (2.0 g., 0.01 mole) in 30 ml. of dry benzene was then added drop by drop to the suspension, which was then gently warmed for 5 min. A reddish-yellow precipitate formed. The reaction mixture was allowed to stand 2 hr. at room temperature with constant stirring. Hydrolysis of the reaction mixture was effected with 100 ml. ice-cold water, and the resulting organic layer was washed successively with distilled water (30 ml.) and with aqueous 10% NaOH solution (30 ml.). The combined aqueous extracts were washed with ether (2 x 25 ml.) and then acidified with dil HCl (pH = 6). A yellowish-brown puffy substanced formed and was filtered off. It was washed several times (6 x 25 ml.) with distilled water and air-dried to give 2.2 g. (96.1%) of 98, m.p. 151°. The molecular weight was determined by mass spectral analysis to be 220.

Anal. Calcd. for $C_{12}H_{12}O_4$: C, 65.45; H, 5.45.

Found: C, 65.37; H, 5.39.

NMR spectral analysis (Plate XVIb) confirmed the proposed structure for 98.

In another preparation the reaction was carried out under the same conditions (compare with 95) except for a longer reaction time (about

12 hr.). The yield remained unchanged, but the purity of sample was less (m.p. 145-147°).

Preparation of N-Methyl-6-methoxy-1-tetralone imine (86). To a dry, nitrogen-purged, four-necked, 500-ml. reaction vessel equipped with an addition funnel, dry-ice condenser, mechanical stirrer, and gas inlet tube was added a solution of 6-methoxytetralone (10.0 g., 0.057 mole) in 125 ml. of anhydrous ether. The reaction vessel was cooled to below -18° (ice-methyl alcohol mixture) and an excess of anhydrous methylamine (25 ml.) was distilled into the reaction flask. Methylamine was already liquified by cooling the gas to -18° (ice-methanol).

A solution of titanium tetrachloride (5.7 g., 0.03 mole) in 100 ml. of dry n-pentane was added dropwise with stirring over a 1-hr. period. ($TiCl_4$ was weighed under N_2.) After the addition was complete, the reaction mixture was stirred at room temperature for 1 hr. It was then diluted with 100 ml. of dry ether and filtered by suction. The filtrate was concentrated (50 ml.) and cooled in the regrigerator overnight. A crystalline solid formed and was filtered off, washed several times (6 x 30 ml.) with ice cold anhydrous n-pentane, and dried under vacuum to give 10.5 g. (95.85%) of 86 (m.p. 53-55°). The molecular weight found by mass spectral analysis was 189.

Anal. Calcd. for $C_{12}H_{15}NO$: C, 76.19; H, 7.93; N, 7.40.

Found: C, 76.36; H, 8.14; N, 7.24.

NMR and IR spectral data (Plates XXIIb and IVb) confirmed the proposed structure.

General Procedure for Investigation of Molecular Complexes. Compounds under investigation were prepared in our laboratories and

purified by either recrystallization or by sublimation. In each case the proposed structure was confirmed by NMR, IR, and mass spectral data along with elemental analysis prior to use in the complexation studies.

The ultraviolet absorption measurements (on a Cary 14 spectrophotometer) of the molecular complexes and individual components were performed using quartz cells of 1 mm.; and the NMR studies were carried out using 5 mm. (O.D.) tubes maintaining the spin rate at 30 r.p.s.

Relatively concentrated solutions were first prepared (for UV studies) by accurately weighing out the appropriate samples in separate glass-stoppered, 50 ml. volumetric flasks and diluting to volume with 95% ethanol. The final solutions were then prepared by pipeting a portion of this solution into a separate volumetric flask and diluting to the desired volume. The concentrations used are given in Tables XV and XVIII. The NMR solutions were made up by dissolving accurately weighed appropriate samples in suitable deuteriated NMR solvents. All runs and measurements were repeated for the sake of completeness and deviation was less than 2% of the absolute values obtained.

A. Molecular Complexation Studies Between 1,4-Dihydro[1]benzothiopyrano[4,3-c]pyrazole (65) and 1,3,5-Trinitrobenzene (121). The following procedure is characteristic of that employed for the compounds 65 and 121. The general procedure described above was followed to obtain NMR and UV spectral data (Tables XIV and XV) on the mixture as well as of the unbound (free) reactants. Certain key regions in the NMR spectra of the mixture and of the corresponding regions in the spectra of the free components have been expanded for the sake of clarity and improved resolution.

A sample of the compound 65 (0.0453 g., 2.409×10^{-4} mole) was dissolved in 1 ml. of acetone-d_6 (99.5%) with a resulting concentration of 2.409×10^{-1} mole/liter. An equimolar solution of the acceptor (0.0513 g., 2.409×10^{-4} mole) was prepared in a similar fashion. A 1:1 mixture of the compounds 65 and 121 was also made following the same procedure, and the solutions were then examined via NMR analysis (Table XIV). Ultraviolet absorption maxima for the individual compounds and the resulting mixture in 95% ethanol (9.63×10^{-5} mole/liter each) were also determined (Table XV).

The formation of a molecular complex was deduced from the NMR data, which included chemical shift differences (Δv) derived by comparison of the spectra of the unbound reactants and complexed reactants. A reasonable structure for the complex consistent with the observed shift trends and intensity was then postulated (Figure 10).

The evaluation of the association constant, K, was carried using the Hanna and Ashbaugh method.[116] For reasons of availability of material and solubility of same, concentration of the acceptor candidate was used in excess.

The association constant, K, was calculated using the equation

$$\delta D_{obs} - \delta D_o = \frac{[A]K}{1 + [A]K} (\delta D_{AD} - \delta D_o) \qquad (1)$$

where δD_{obs} = observed chemical shift of the selected donor protons in the complexing medium; δD_o is the chemical shift of selected donor protons in the uncomplexed state; and δD_{AD} is the shift of selected donor protons in the pure complex. A simplified version of the above equation assumes the form (2) as shown below:

$$\Delta_{obs} = \frac{[A]K}{1 + [A]K} \Delta_o \qquad (2)$$

where

$$\Delta_{obs} = \delta D_{obs} - \delta D_o$$

and

$$\Delta_o = \delta D_{AD} - \delta D_o.$$

The reciprocal of equation (2) gives a new form which is represented by (3)

$$\frac{1}{\Delta_{obs}} = \frac{1}{K\Delta_o} \frac{1}{[A]} + \frac{1}{\Delta_o} \qquad (3)$$

To obtain the value of K, a plot was made (Figure 11) of $\frac{1}{\Delta_{obs}}$ versus $\frac{1}{[A]}$. From the y-intercept and slope obtained from the graph, the association constant, K, was computed and found to be 1.74 ℓ/m, which is in good agreement with the reported K values of related systems such as the N,N-dimethylaniline-1,4-dinitrobenzene complex (K = 0.05 ℓ/m) and the pentamethylbenzene-1,3,5-trinitrobenzene complex (K = 1.93 ℓ/m).

The general procedure was used to investigate molecular complexes for the following systems as in the previous procedure A.

B. The molecular complex involving 1,4-dihydro[1]benzothiopyrano-[4,3-c]pyrazole (65) and 4-fluorophenylacetonitrile (122).

C. The molecular complex involving pyrazole 65 and 3,4-dihydroxy-benzoic acid (123). In this case, the temperature dependence of the spin-spin relaxation times of the mixture was analyzed by measuring the width of the resonance signal at half height ($\Delta V_{1/2}$) and using the

relationship $T_2 = \frac{2}{\Delta V_{1/2}}$ sec. (Table XIX).

D. The molecular complex between pyrazole 65 and 5-fluorouracil (42).

E. The molecular complex between 4H-[1]benzothiopyrano[3,4-d]-isoxazole (68) and 5-fluorouracil (42).

F. The molecular complex between 4,5-dihydro-6,7,8-trimethoxy-1H-benz[g]indazole (132) and 5-fluorouracil (42).

PMR analysis of these systems reveal a preferred organization of both the donor and acceptor in the complexes. The nature of the complex and the factors influencing the complexation process have been discussed (Discussion, pages 110-132).

A Study of the Spectra and Acidity of 4,5-Dihydro-1H-benz[g]-indazol-7-ol (82) and 2,3a,4,5-Tetrahydro-7-hydroxy-3a-methyl-3H-benz-[g]indazol-3-one (80). All experimental pH measurements were made on a Beckman 101900 research pH meter with a readability of 0.005 pH units. The glass electrode used was Beckman 39301 electrode. As external reference electrode, a Beckman calomel electrode was employed. The titration was carried out using a Gilmont ultraprecision micrometer burette. The pH meter was standardized employing "pHydrion buffers" of pH 6.86 and 9.4.

The compound 80 (6.0×10^{-3} g.; 2.78×10^{-5} mole) was carefully weighed and dissolved in 28.90 ml. of 1.443×10^{-3} N NaOH. This solution was degassed with N_2 for 15 min. prior to titration, and then it was titrated against standard 2.780×10^{-2} N HCl. A magnetic stirrer was used during the titration. For indazolone 80, the pK_a value was found to be 8.7.

Indazole 82 (5.2×10^{-3} g.; 2.78×10^{-5} mole) was carefully weighed and dissolved in 21.7 ml. of 1.443×10^{-3} N NaOH and diluted to 200 ml. with doubly distilled water. This solution also was degassed with N_2 for 15 min. prior to titration, and then it was titrated against 5.56×10^{-2} N HCl. For the compound 82, the pK_a value was found to be 9.1.

Indazole 82 (1.12×10^{-4} mole/liter) in water was used for the purpose of UV spectral studies. UV spectra at 3 different pH values: 3.2, 6, and 9.6 were analyzed. The ultraviolet absorption data (Tables XII and XIII) for 80 and 82 are interpreted as supportive of the proposed tautomeric structures.

Attempted Preparation of 3-Benzyloxyacrylonitrile (124). Cyanoacetylene (10.0 g., 0.196 mole) in 100 ml. of anhydrous ether was stirred into a suspension of 4.10 g. (0.379 mole) of benzyl alcohol and 5.0 g. atom) of sodium metal in 100 ml. of dry ether under dry ice-methanol cooling system (-75°). The reaction mixture was stirred (magnetic stirrer) overnight at room temperature under N_2. A black-colored solid appeared as soon as cyanoacetylene was added dropwise. After stirring overnight under N_2, the solvents were removed by evaporation (rotary evaporator). The residue was a black tarry substance. Attempts were made to recrystallize it but to no avail. Formation of a polymeric substance was inferred as suggested by its insolubility in many readily available organic solvents.

Attempted Preparation of 7-Methoxy-2,3a,4,5-tetrahydrobenz[g]indol-3-one (125). To a solution of 6-methoxytetralone (5.28 g., 0.03 mole) and ethyl α-aminoacetate hydrochloride (4.185 g., 0.03 mole) in 100 ml. of toluene was added 6.72 g. (0.06 mole) of potassium t-butoxide and

the reaction mixture was stirred (magnetic) at room temperature. A reddish, powder formed an an exothermic reaction ensued with a sudden rise in temperature of the reaction mixture (to ∿ 40°). It was then heated gently to 80° for 0.5 hr. and cooled to room temperature. Acidification (conc. HCl) was performed while cooling the flask in an ice bath; the solution was then extracted (ether, 3 x 25 ml.). Both layers were separately chilled in a refrigerator overnight. No precipitate formed.

A small portion of the nonaqueous layer was treated with n-hexane and cooled in ice. A brown solid formed and was filtered out by suction, washed several times (water, 6 x 25 ml.), and air-dried to give 0.6 g. of a product. NMR analysis of this solid revealed an unexpected substance of very high melting point (m.p. > 325°).

Interestingly, the aqueous layer turned red in acid medium, green-brown in neutral medium, and yellowish-brown in basic medium. No identifiable solid product could be isolated from the reaction mixture.

Attempted Preparation of 7-Methoxy-1-methyl-4,5-dihydrobenz[g]indol-3-(2H)-one (126). To a dry, nitrogen-filled, 3-necked flask equipped with a condenser and serum cap was added 5.93 g. (0.0314 mole) of the imine 86 in 6 ml. of THF. Isopropylmagnesium chloride (40 ml.) in THF was then slowly added to the reaction mixture via a syringe at a rate which maintained a gentle reflex. To the resulting mixture was added 4.52 g. (0.04 mole) of chloroacetyl chloride at such a rate that boiling was maintained. The reaction mixture turned blood-red color. On completion of the addition of chloroacetyl chloride, an additional 20 ml. of the Grignard reagent was added to the mixture all at one time.

The reaction mixture was then diluted with 50 ml. of 1.0 M. aqueous solution of EDTA tetrasodium salt and 150 ml. of 1:1 ether-benzene solution. The organic layer was separated by extracting with water (3 x 25 ml., found to be basic). The process of extraction was continued until the solution was neutral. The separated organic layer was finally extracted with saturated NaCl and dried (Na_2SO_4) overnight. The solution was then filtered by suction, the solvent was removed from the filtrate on a rotary evaporator, and the concentrate stored in the refrigerator. No crystals formed. GLC analysis showed only two compounds--solvent (benzene) and the product.

Attempted Preparation of 1,4-Dihydro-6,7-dimethoxyindeno[1,2-c]-pyrazole (127). Hydroxymethylene compound 98 (0.5 g., 0.0023 mole) was dissolved in 40 ml. of absolute methanol, and to this solution was added 1 ml. of 97% hydrazine with constant stirring (magnetic stirrer) under N_2. The reaction mixture turned from yellow to orange color and was stirred (6 hr.) at room temperature. It was then diluted with 75 ml. of cold distilled water, boiled for 0.5 hr., and cooled to room temperature. Upon cooling in ice, orange crystals formed; these were filtered off under suction and air-dried to give 0.1 g of 127 (m.p. 211-212°). The molecular weight determined by mass spectral analysis was 464 instead of the proposed molecular weight of 216 (Calcd. for $C_{12}H_{12}N_2O_2$). Due to its severe insolubility in organic solvents an NMR spectrum of the compound 127 could not be obtained without excessive time averaging (estimate one to two weeks without Fourier transform and pulse equipment).

Attempted Preparation of 6,7-Dimethoxy-4H-indeno[2,1-d]isoxazole (128). To a solution of 2-hydroxymethylene compound 98 (0.5 g.,

0.0023 mole) in 35 ml. of glacial acetic acid was added 0.207 g. (0.003 mole) of hydroxylamine hydrochloride in 5 ml. of distilled water. This solution was heated gently for 0.5 hr. with stirring (magnetic stirrer) and then cooled to room temperature. After stirring (magnetic) overnight, the mixture was tirturated with cold distilled water. The dark-red crystals formed and were filtered off under suction and air-dried to give 0.3 g. of 128 (m.p. 225-228° with shrinking at 218°). The molecular weight found by mass spectral analysis was 467, but the expected molecular weight (Calcd. for $C_{12}H_{11}NO_3$) for 128 was 217. Again, NMR spectral analysis was not rewarding owing to the extreme insolubility of the product.

Attempted Preparation of Pyrazolone of Thiochroman-e-one (129). Thiochroman-4-one (90) (5.3 g., 0.0323 mole) and 1.89 g. (0.035 mole) of sodium methoxide were dissolved in 75 ml. of anhydrous dimethyl carbonate (distilled with NaH). The reaction mixture was boiled under N_2 for 3 hr. The mixture turned red within 5 min. It was cooled to room temperature and then a portion of it (30 ml.) was acidified (dil HCl). No precipitate formed. This solution was concentrated to a small volume (15 ml.) by evaporation (rotary evaporator) and cooled (ice/H_2O bath). It was then filtered under suction and a black tarry substance was isolated. Attempts were made to dissolve it in acetone. The part insoluble in acetone was then filtered off by suction and washed several times with cold distilled water. It was air-dried to give a yellow-colored solid (2.2 g., m.p. 215-217°). The molecular weight determined by mass spectral analysis was 534 instead of the expected molecular weight 222 for the keto ester intermediate for the preparation of pyrazolone 129.

Because of the extreme insolubility of this solid in common organic solvents, an NMR spectrum of the aforementioned material could not be obtained in a single solvent. Fortunately the solid was found to be partially soluble in pyridine-d_5. A drop of CF_3CO_2H was added to the mixture to increase the solubility. Interestingly, a white, powdery solid formed and was filtered off by suction. The white solid was insoluble in both organic as well as inorganic solvents. The same substance formed when pyridine and CF_3CO_2H were mixed (an exothermic reaction ensued with a great evolution of heat). The white powdery substance formed was found to be very similar to teflon in appearance and solubility.

Since the compound could not be characterized as the expected keto ester intermediate, further attempts to cyclize it to the pyrazolone 129 were not pursued.

Attempted Preparation of N,N-Dimethyl-6-methoxytetralone iminium iodide (130). Imine 86 (0.5 g., 0.0027 mole) was dissolved in 50 ml. of anhydrous ether and to this solution was added 2 ml. of methyl iodide. The solution was then boiled for 24 hr. A precipitate formed and was filtered off, washed with ether, and air-dried to yield 0.06 g. of a solid (m.p. 198.5-199°).

An aqueous $AgNO_3$ solution gave a yellow precipitate, which indicated the presence of iodide ion. The elemental analysis was unsatisfactory. No further work was done.

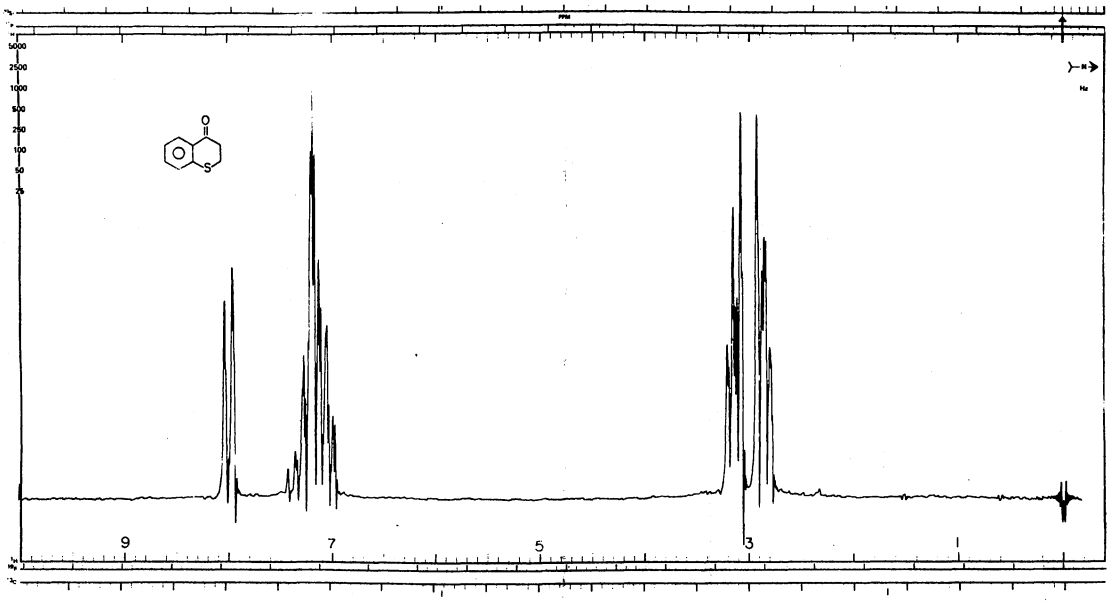
Anal. Calcd. for $C_{13}H_{18}NO$: C, 47.13; H, 5.44; N, 4.23.

Found: C, 44.56; H, 4.91; N, 4.52.

Attempted Preparation of 3,4-Dihydro-3-ethoxy-3-(6-methoxy-1-oxo-(2H)-2-naphthyl)propionic acid (131). To a magnetically stirred solution

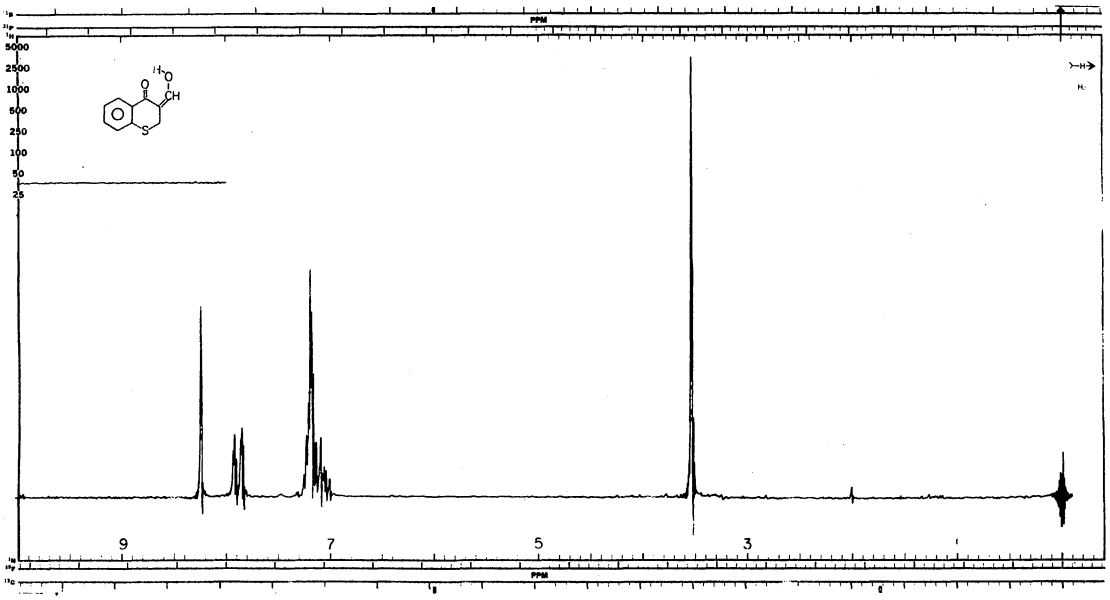
of 6-methoxytetralone (7.6 g., 0.043 mole) in 125 ml. of dry THF was added 4.0 g. (0.08 mole) of sodium methoxide. A solution of 8.0 g. (0.06 mole) of ethoxymethylene malononitrile in 25 ml. of THF was then added. As the reaction progressed, the mixture darkened. After boiling for 15 min. heating was stopped, but stirring was continued overnight. To the resulting reaction mixture was added ether (50 ml.) and the solution was filtered by suction. The solid obtained was redissolved in water which was acidified (dil HCl) (pH = 6). A precipitate formed and was filtered off and air-dried to give 2.0 g. (m.p. 200-214°) of a solid. This solid was then magnetically stirred into 100 ml. of 95% ethanol, and to this mixture was added excess NaOH (15.0 g.) dissolved in 100 ml. of distilled water. The resulting reaction mixture was boiled for 100 hr. Evolution of NH_3 gas was indicative of the progress of reaction. It was finally diluted with distilled water and washed with ether (3 x 25 ml.). The water layer was then acidified (dil HCl) and chilled in the refrigerator. A solid formed and was filtered off, washed several times with distilled water and air-dried to weigh 0.8 g. (m.p. > 340°). Attempted purification and characterization of the compound did not prove fruitful owing to its extreme insolubility in essentially all solvents used.

PLATE I



Ia. Thiochroman-4-one (90)

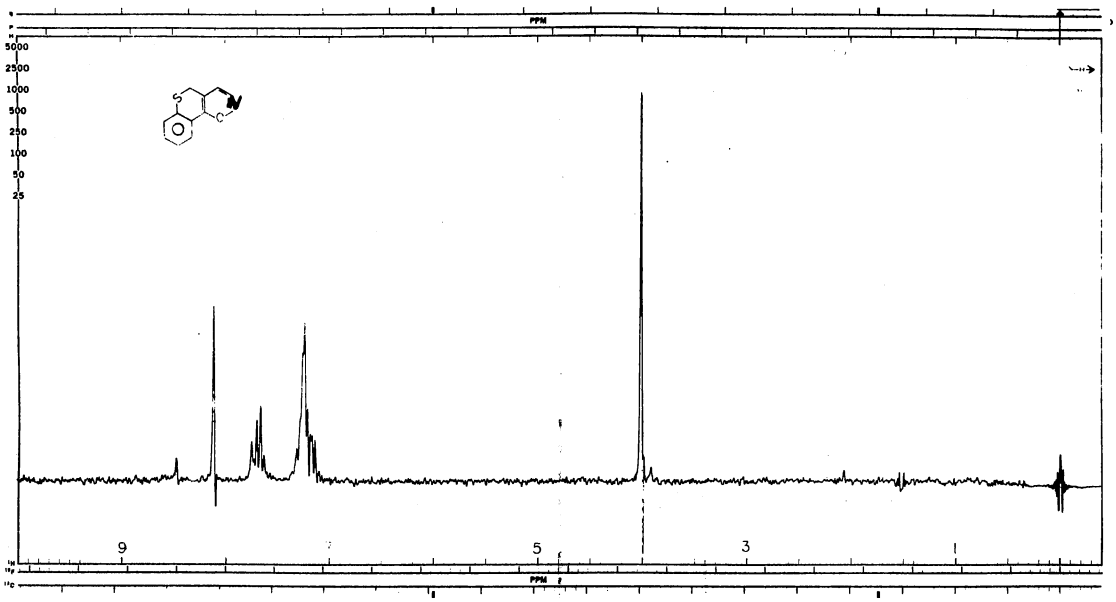
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . 2 R.F. . . 66 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . .83701 Hz S.A. . . .1.25
Lock . . . . HOMO



Ib. 2(Hydroxymethylene)thiochroman-4-one (94)

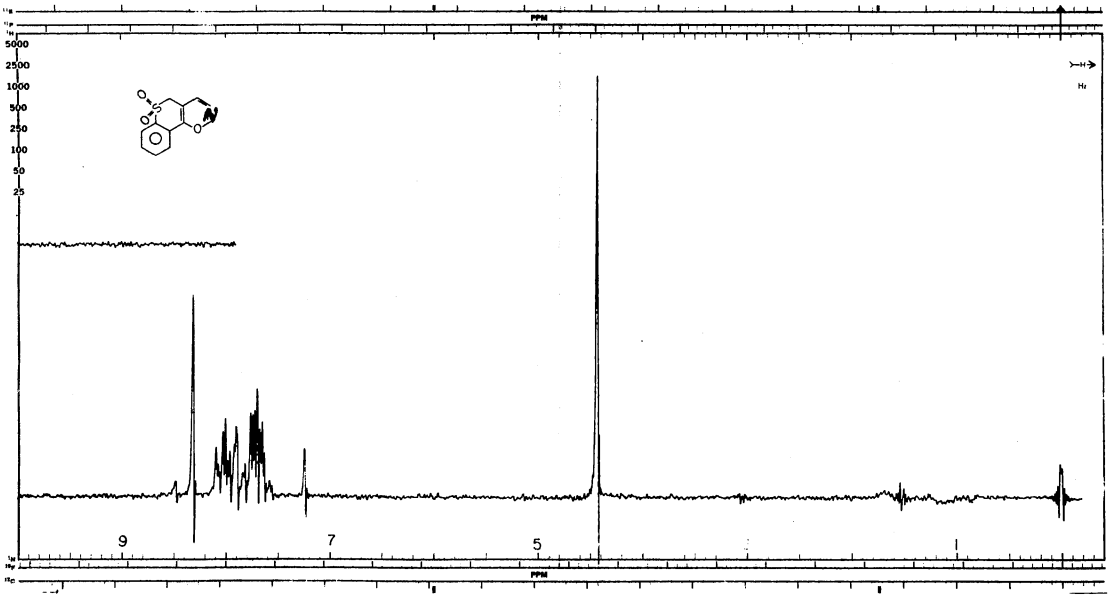
Solvent. . .$DCCl_3$ O.F. . 100.1 MHz F.B. . . . . 2 R.F. . . 60 dB
S.W. . . .1000 Hz S.T. . . 500 Sec S.O. . 83701 Hz S.A. 1
Lock . . . . HOMO

PLATE II



IIa. 4H-[1]Benzothiopyrano[3,4-d]isoxazole (68)

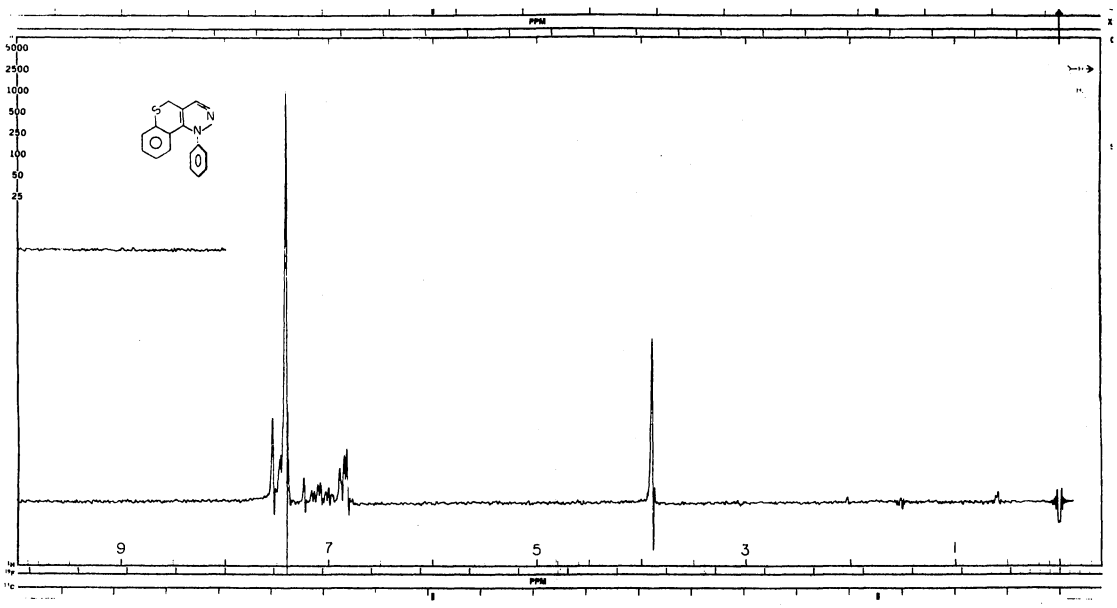
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 62 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. . . . 6.3
Lock . . . . HOMO



IIb. 4H-[1]Benzothiopyrano[3,4-d]isoxazole 5,5-dioxide (72)

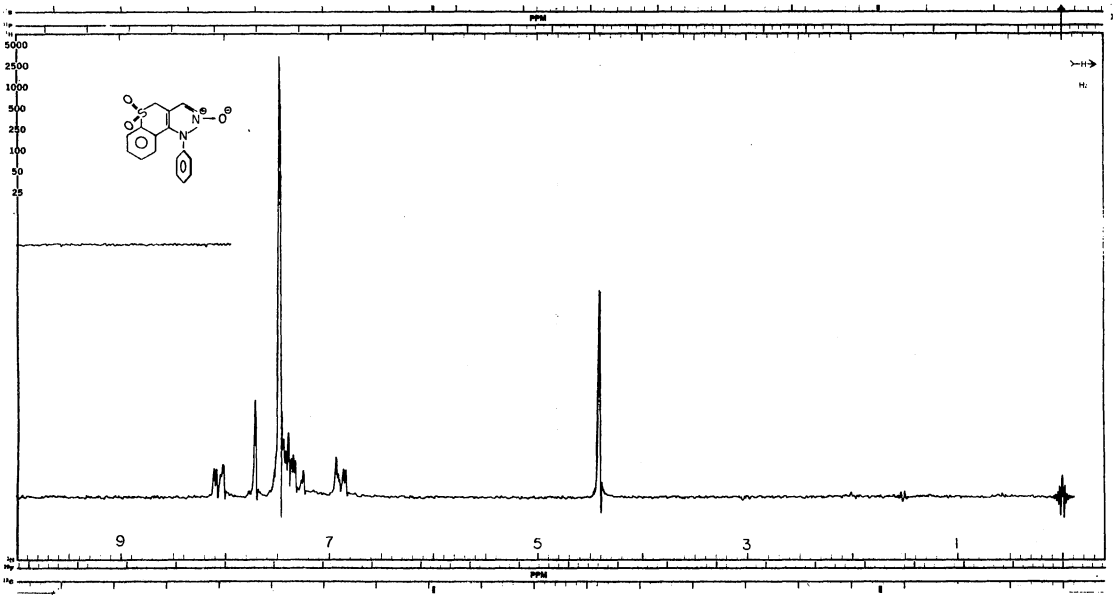
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 69 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. 5
Lock . . . . HOMO

PLATE III



IIIa. 1,4-Dihydro-1-phenyl[1]benzothiopyrano[4,3-c]pyrazole (66)

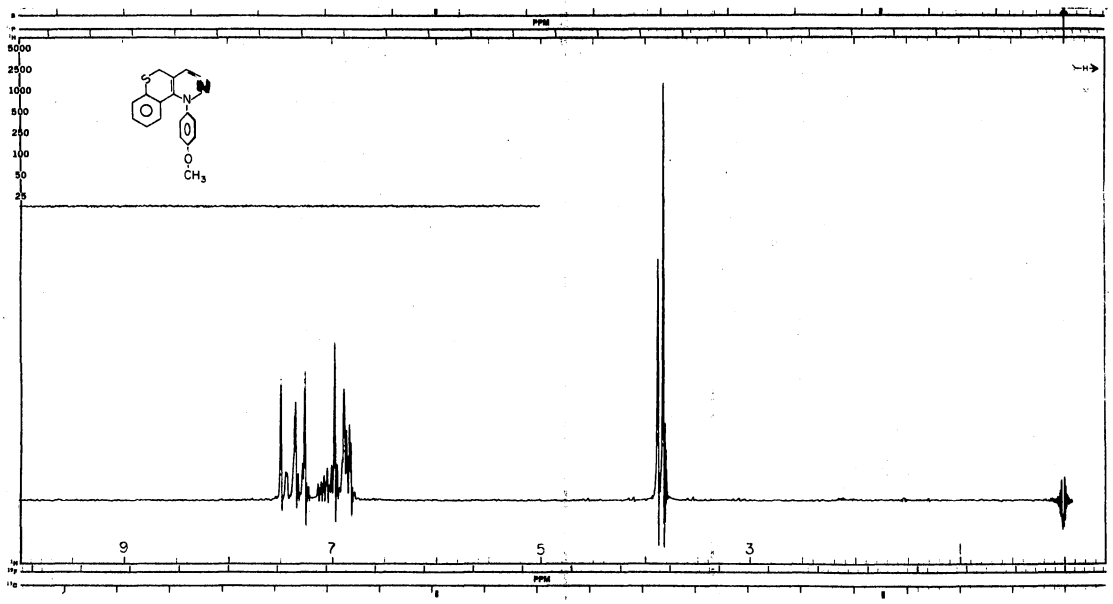
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 66 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. . . . 3.2
Lock . . . . HOMO



IIIb. 1,4-Dihydro-1-phenyl[1]benzothiopyrano[4,3-c]pyrazole-2,5,5-trioxide (71)

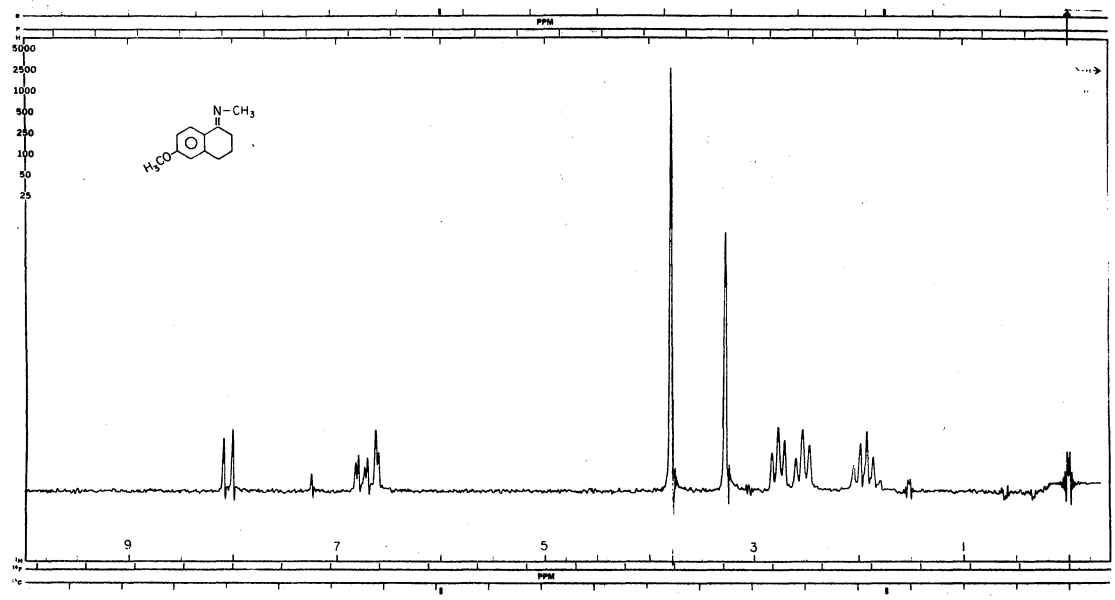
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 67 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. . . . 2.5
Lock . . . . HOMO

PLATE IV



IVa. 1,4-Dihydro-1-(p-methoxyphenyl)-[1]benzothiopyrano[4,3-c]-pyrazole (67)

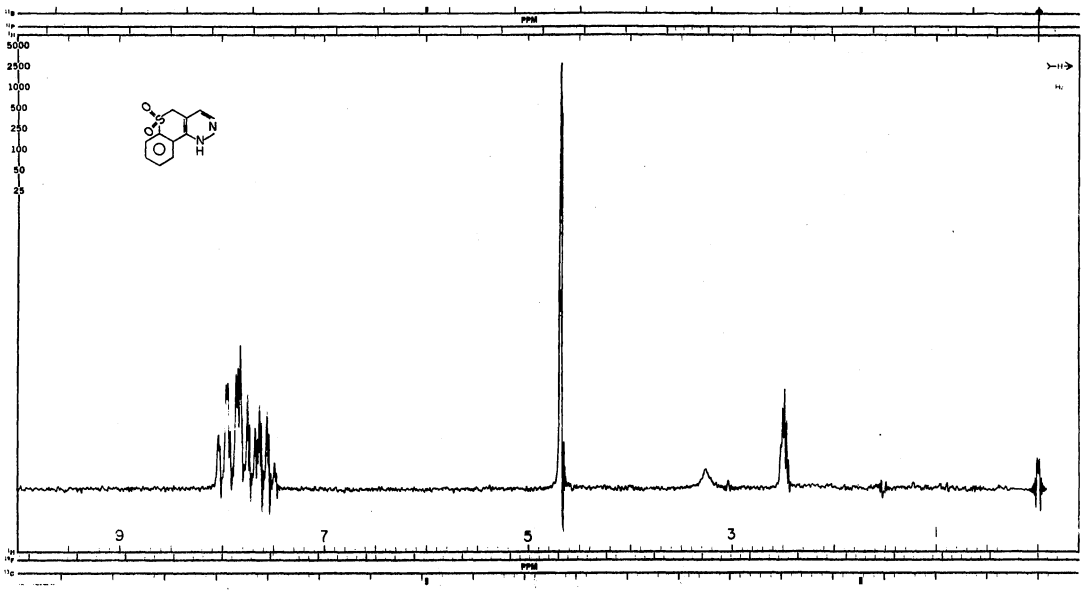
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . .65 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. 8.0
Lock . . . . HOMO



IVb. N-Methyl-6-methoxy-1-tetralone imine (86)

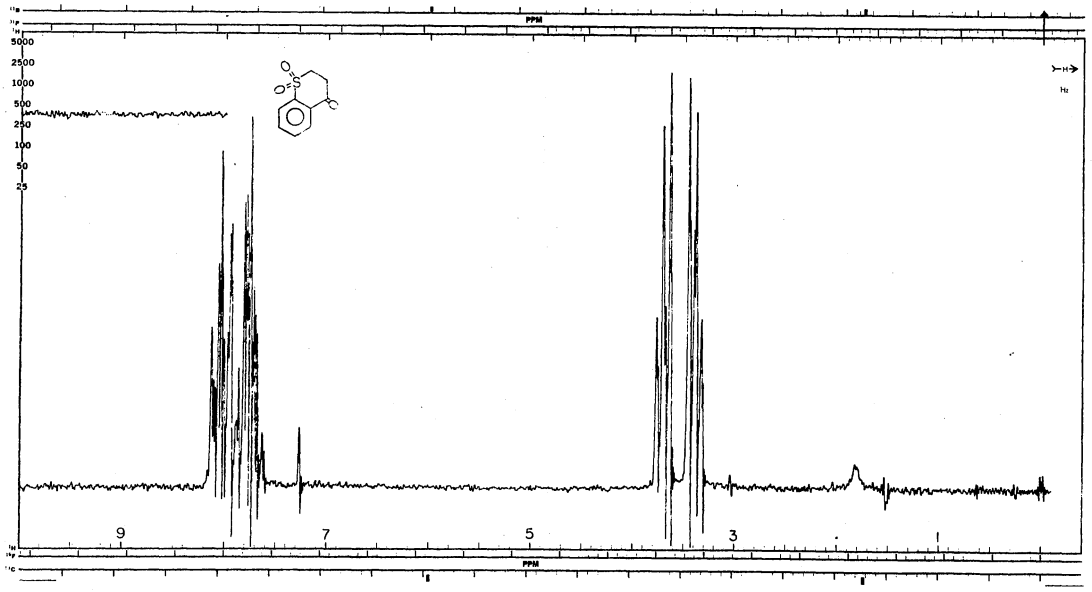
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 65 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. . . . 8.0
Lock . . . . HOMO

PLATE V



Va. 1,4-Dihydro[1]benzothiopyrano[4,3-c]pyrazole 5,5-dioxide (70)

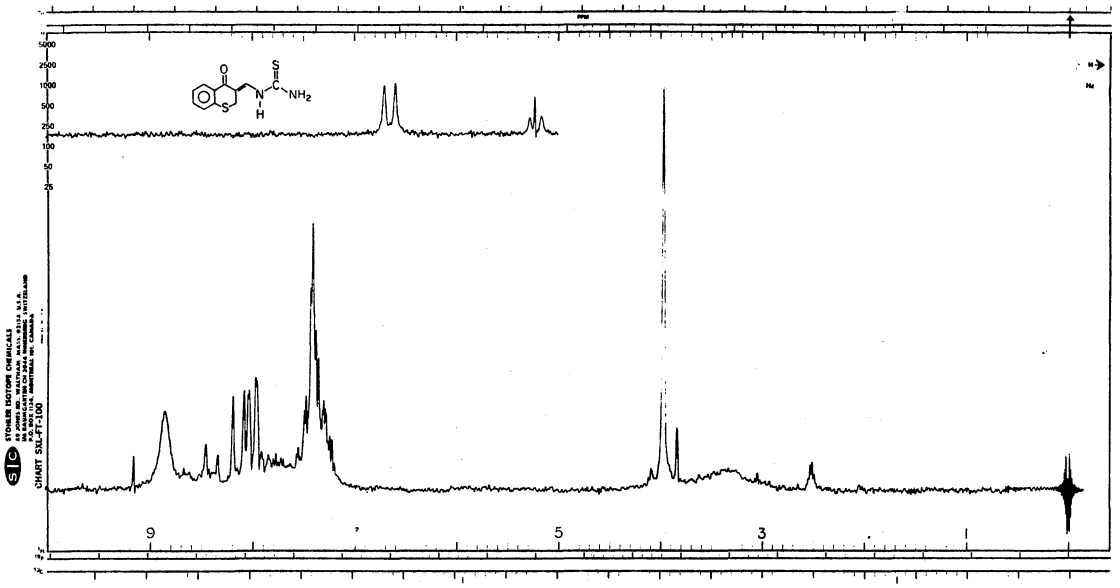
Solvent. .DMSO-d_6 O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 69 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S. A. 5
Lock . . . . HOMO



Vb. Thiochroman-4-one 1,1-dioxide (73)

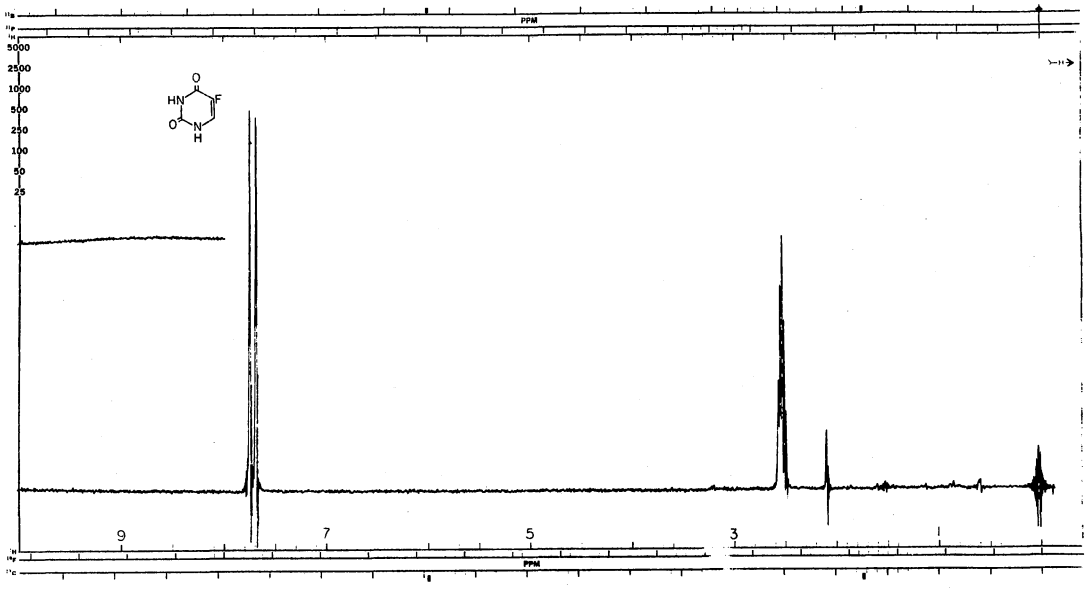
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 66 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. 8
Lock . . . . HOMO

PLATE VI



VIa. 1-[(4-Oxothiochroman-3-yl)methylene]-2-thiourea (78)

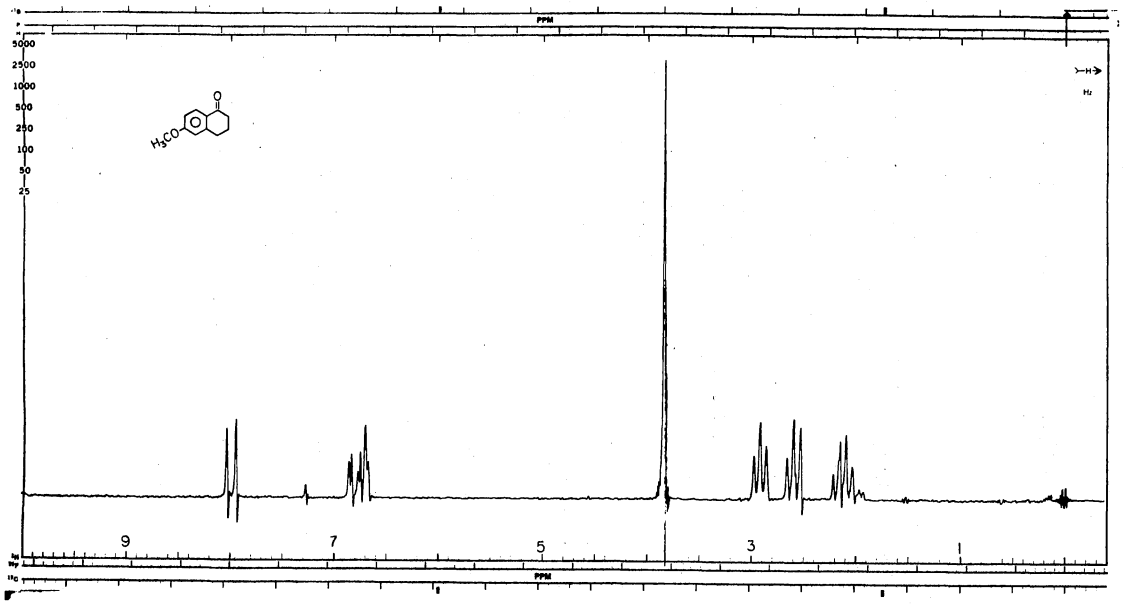
Solvent. .DMSO-d_6 O.F. . .100.1 MHz F.B. . . . . .1 R.F. . . 72 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. . . . 8.0
Lock . . . . HOMO



VIb. 5-Fluorouracil (42)

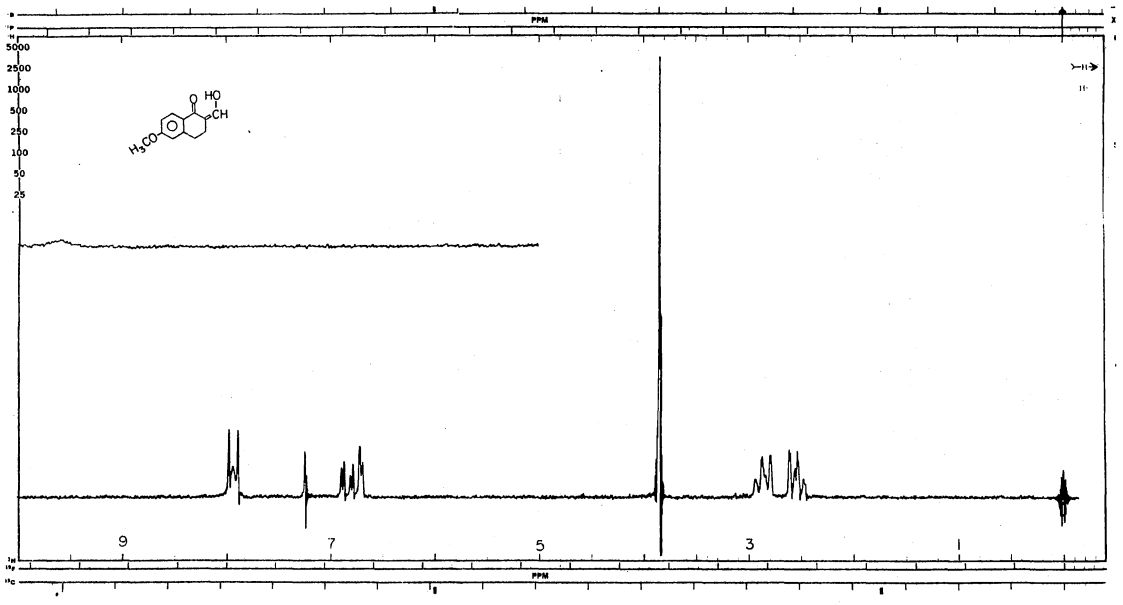
Solvent. .DMSO-d_6 O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 69 dB
S.W. . . .1000 Hz S.T. . . .500 Sec S.O. . . 83701 Hz S.A. . . . 3.2
Lock . . . . HOMO

PLATE VII



VIIa. 6-Methoxy-1-tetralone (89)

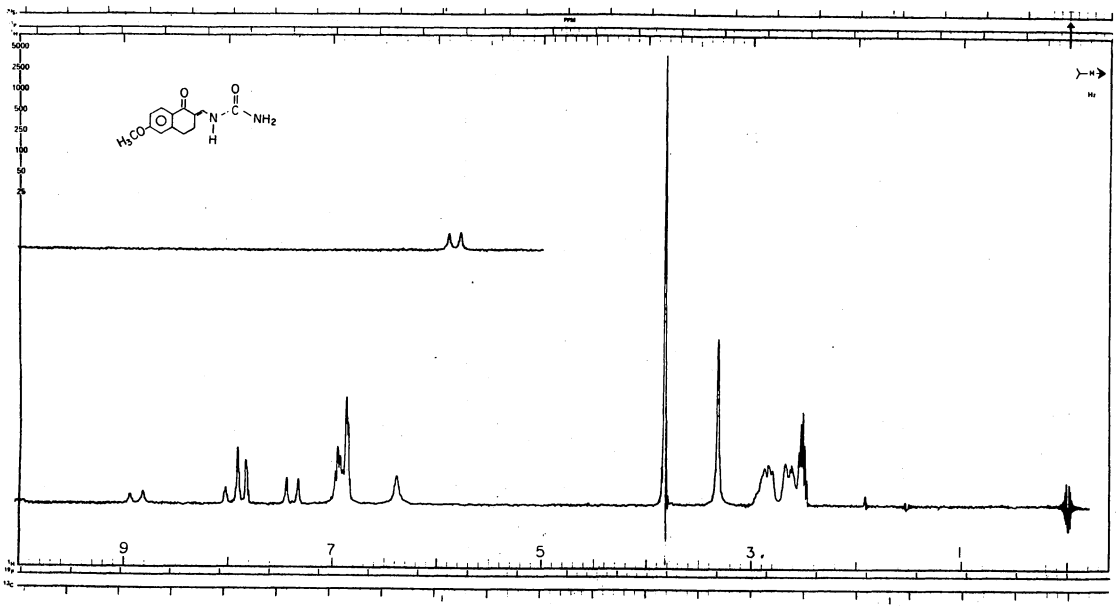
Solvent. . .$DCCl_3$ O.F. . . .100.1 MHz F.B. . . . . .2 R.F. . .65 dB
S.W. . . .1000 Hz S.T. . . . .250 Sec S.O. . . 83701 Hz S.A. . . .1.6
Lock . . . . HOMO



VIIb. 2(Hydroxymethylene)6-methoxy-1-tetralone (93)

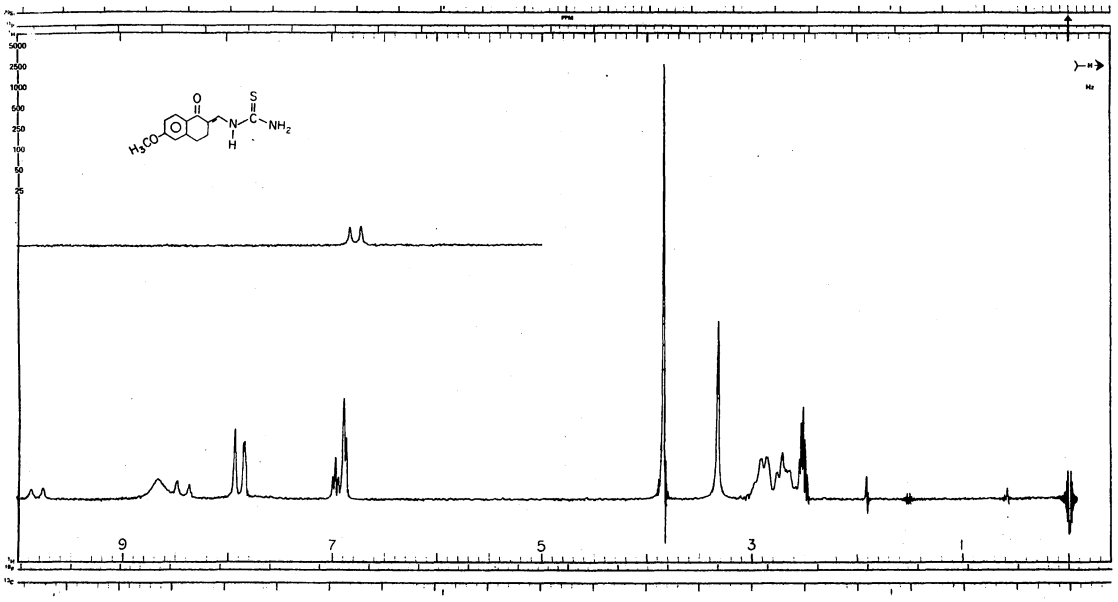
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 66 dB
S.W. . . .1000 Hz S.T. . . . .500 Sec S.O. . . 83701 Hz S.A. . . . 3.2
Lock . . . . HOMO

PLATE VIII



VIIIa. [(1,2,3,4-Tetrahydro-6-methoxy-1-oxo-2-naphthyl)-methylene]urea (76)

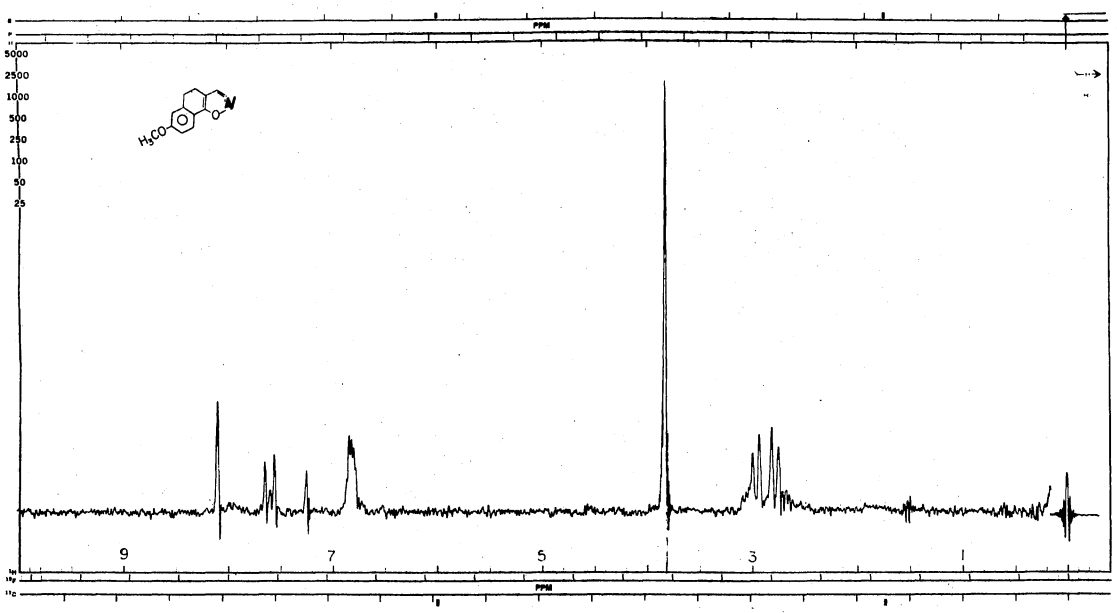
Solvent. .DMSO-d_6 O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 68 dB
S.W. . . .1000 Hz S.T. . . .500 Sec S.O. . . 83701 Hz S.A. 2
Lock . . . . HOMO



VIIIb. [(1,2,3,4-Tetrahydro-6-methoxy-1-oxo-2-naphthyl)-methylene[-2-thiourea (77)

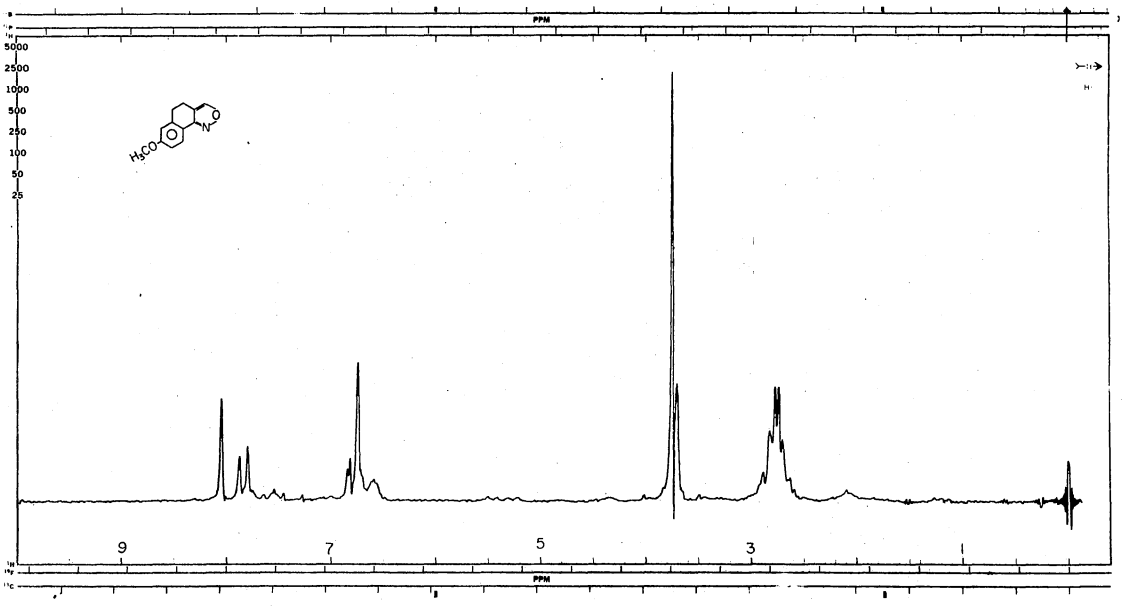
Solvent. .DMSO-d_6 O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 68 dB
S.W. . . .1000 Hz S.T. . . .500 Sec S.O. . . 83701 Hz S.A. 2
Lock . . . . HOMO

PLATE IX



IXa. 4,5-Dihydro-7-methoxynaphth[2,1-d]isoxazole (87)

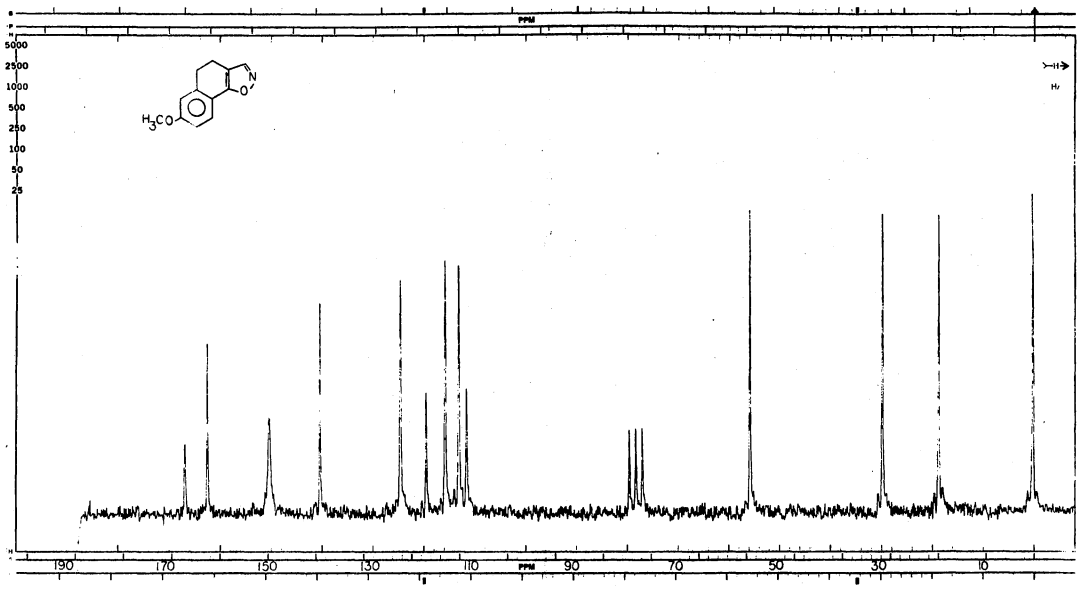
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 66 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. . . .10.0
Lock . . . . HOMO



IXb. 4,5-Dihydro-7-methoxynaphth[1,2-c]isoxazole (88)

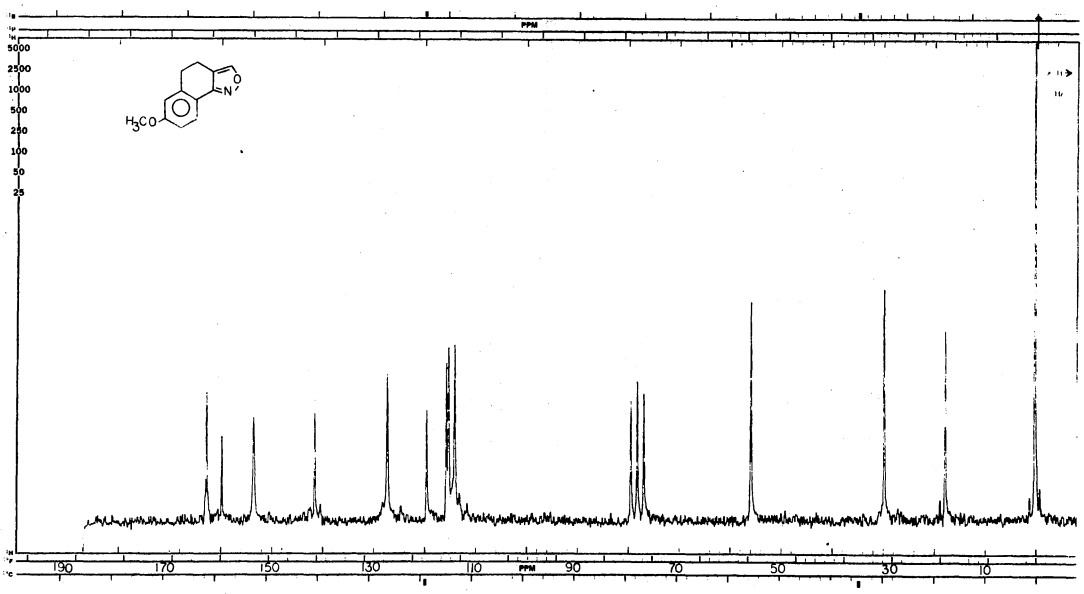
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . .63dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. 1
Lock . . . . HOMO

PLATE X



Xa. ^{13}CMR Spectra of 4,5-Dihydro-7-methoxynaphth[2,1-d]-isoxazole (87)

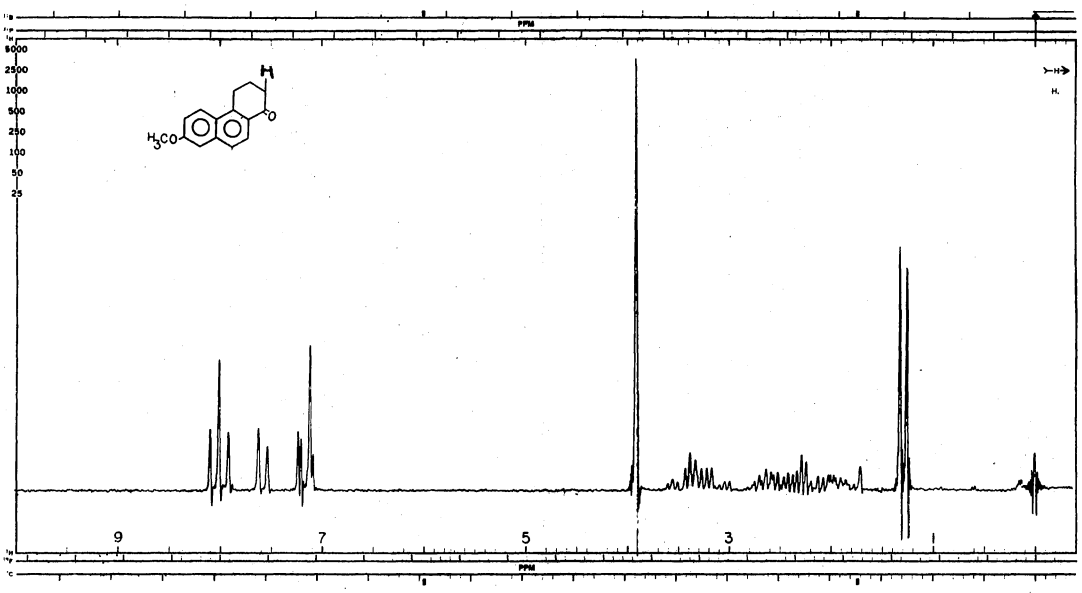
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .4 R.R.F. . . 72 dB
S.W. . . .5000 Hz No. Scans. . .770 S.O. . . 39385 Hz S.A. . . .0.63



Xb. ^{13}CMR Spectra of 4,5-Dihydro-7-methoxynaphth[1,2-c]-isoxazole (88)

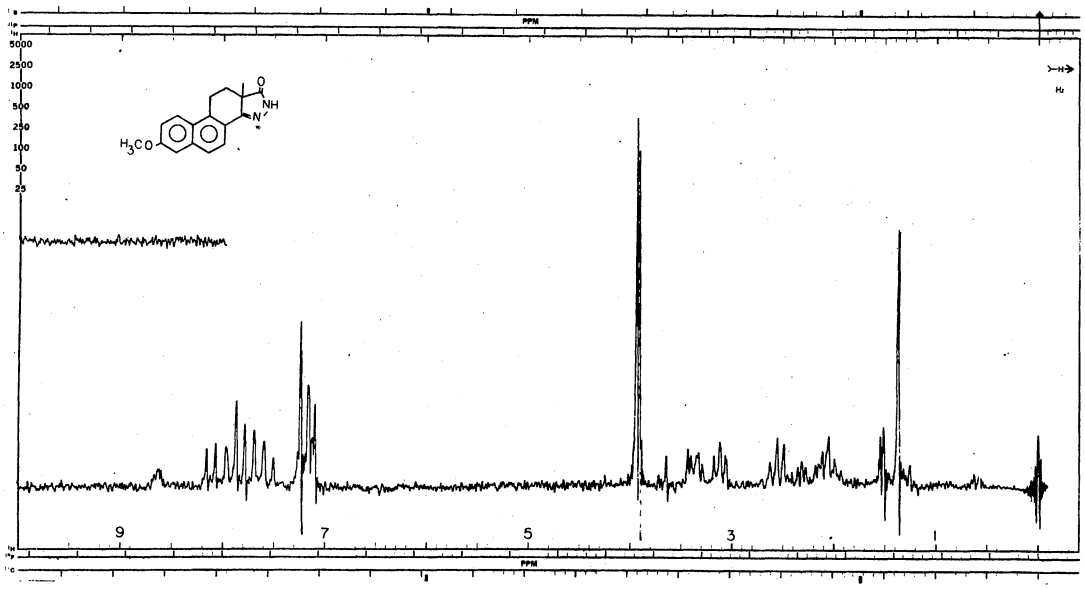
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .4 R.R.F. . . 74 dB
S.W. . . .5000 Hz No. Scans. . 2616 S.O. . . 39385 Hz S.A. . . .0.63

PLATE XI



XIa. 3,4-Dihydro-7-methoxy-1(2H)phenanthrone (101)

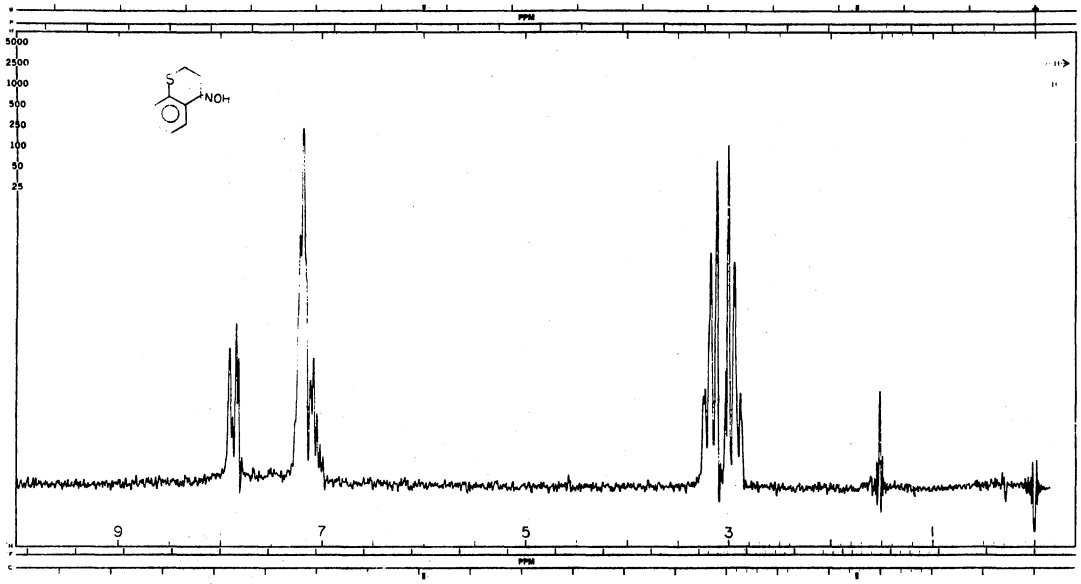
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 69 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. 2
Lock . . . . HOMO



XIb. 2,10,11,11a-Tetrahydro-7-methoxy-11a-methyl-1H-phenanthro[1,2-c]pyrazol-1-one (83)

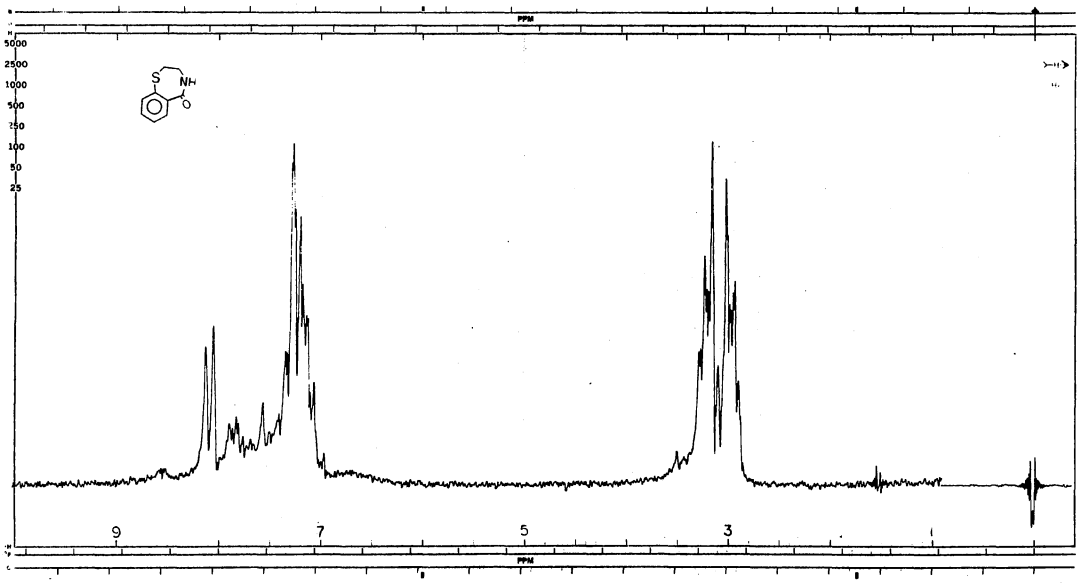
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 71 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. 10
Lock . . . . HOMO

PLATE XII



XIIa. Thiochroman-4-one oxime (74)

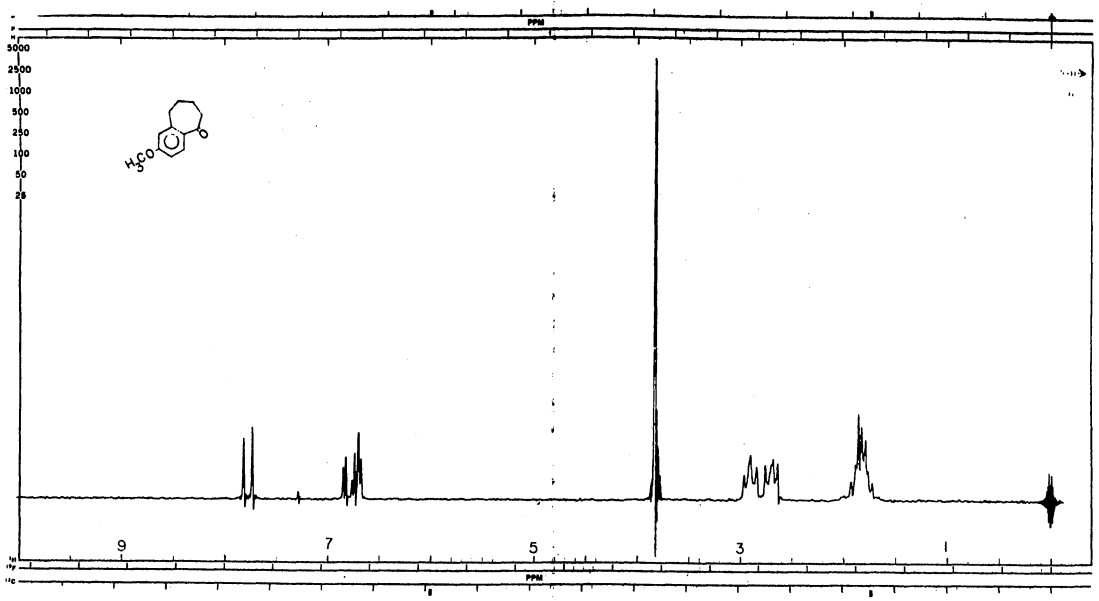
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 69 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. 10
Lock . . . . HOMO



XIIb. 3,4-Dihydro-1,4-benzothiazepin-5-(2H)-one (75)

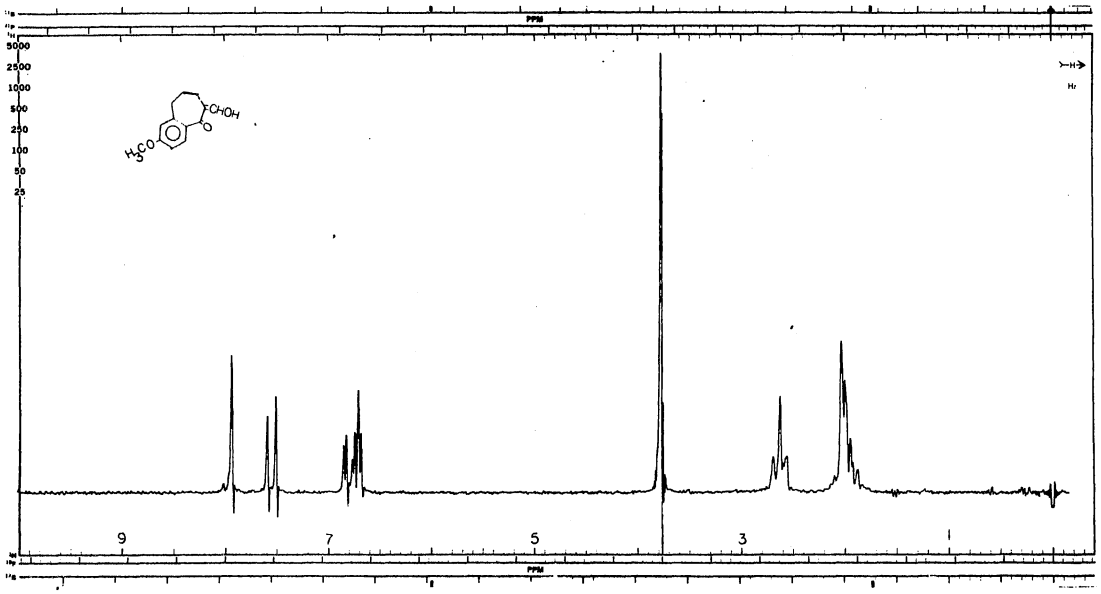
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 68 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. . . . 6.3
Lock . . . . HOMO

PLATE XIII



XIIIa. 2-Methoxybenzosuberone (92)

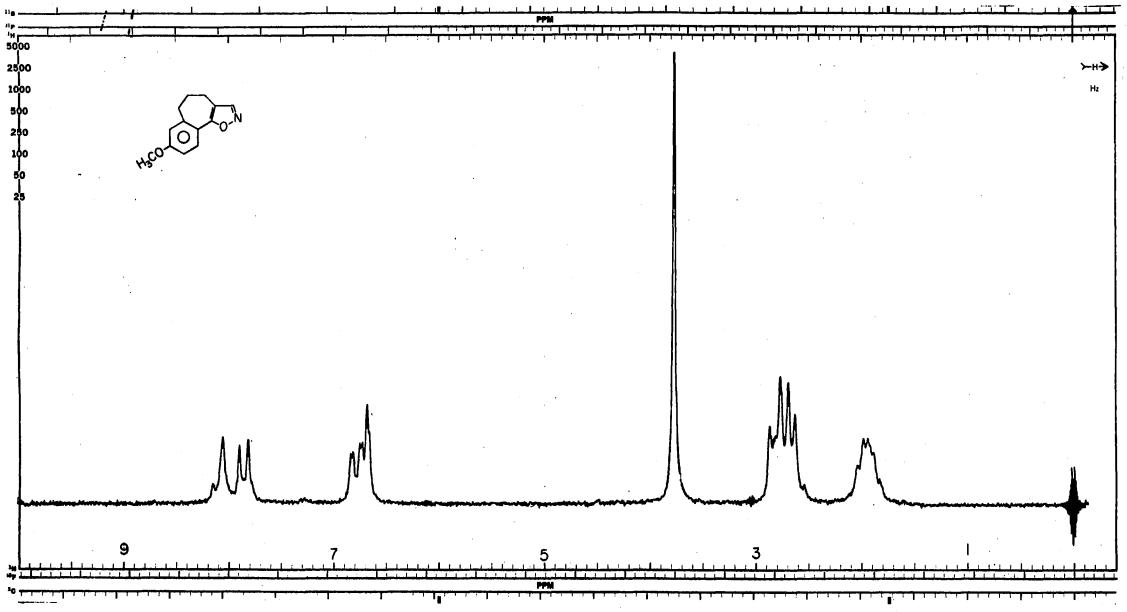
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 60 dB
S.W. . . .1000 Hz S.T. . . .500 Sec S.O. . . 83701 Hz S.A. . . 1.25
Lock . . . . HOMO



XIIIb. 2-(Hydroxymethylene)2-methoxybenzosuberone (96)

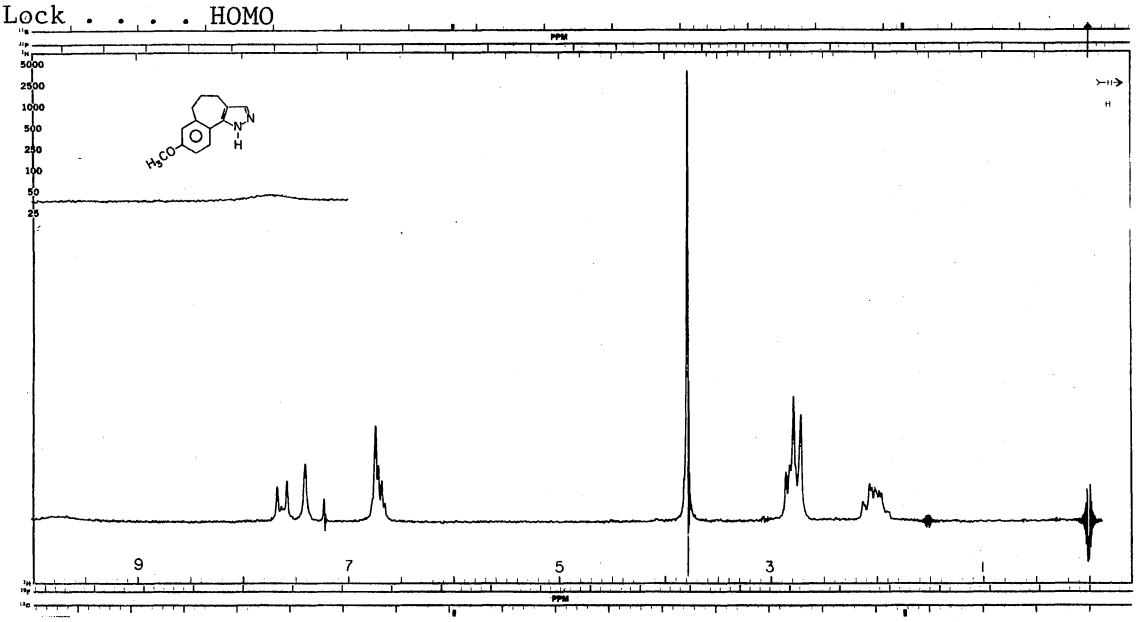
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.F. . . . . .2 R.F. . . 48 dB
S.W. . . .1000 Hz S.T. . . .250 sec S.O. . . 83701 Hz S.A. . . . 3.2
Lock . . . . HOMO

PLATE XIV



XIVa. 5,6-Dihydro-8-methoxy-4H-benzo[3,4]cyclohepta[1,2-d]isoxazole (84)

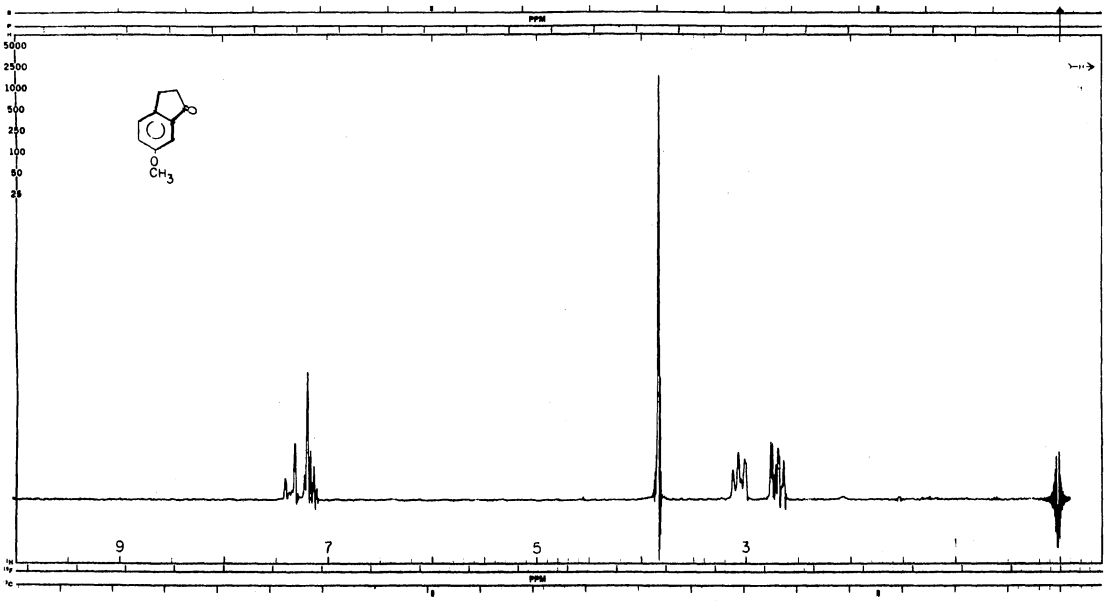
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . .55 dB
S.W. . . .1000 Hz S.T. . . .500 Sec S.O. . . 83701 Hz S.A. 4
Lock . . . . HOMO



XIVb. 1,4,5,6-Tetrahydro-8-methoxybenzo[6,7]cyclohepta[1,2-c]-pyrazole (85)

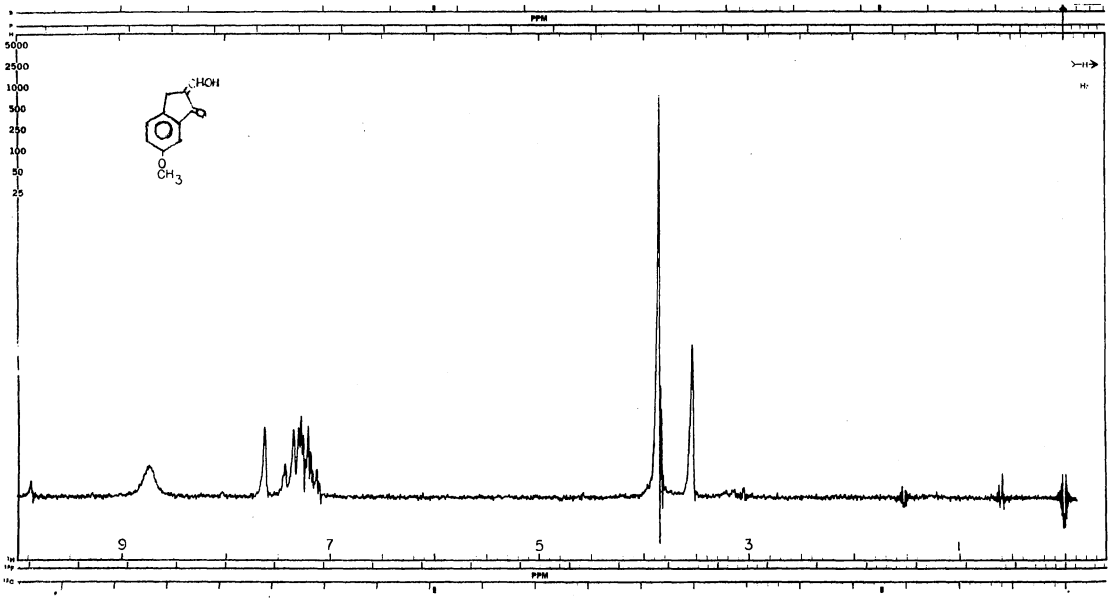
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 66 dB
S.W. . . .1000 Hz S.T. . . .500 Sec S.O. . . 83701 Hz S.A. 2
Lock . . . . HOMO

PLATE XV



XVa. 6-Methoxy-1-indanone (95)

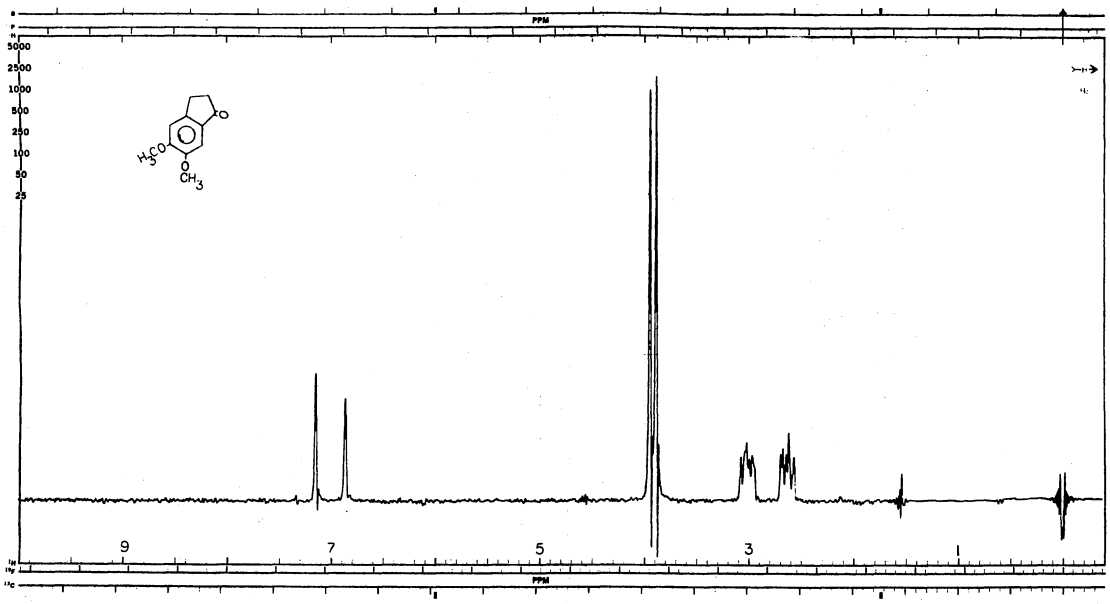
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 60 dB
S.W. . . .1000 Hz S.T. . . .500 Sec S.O. . . 83701 Hz S.A. . . 1.25
Lock . . . . HOMO



XVb. 2-(Hydroxymethylene)6-methoxy-1-indanone (95)

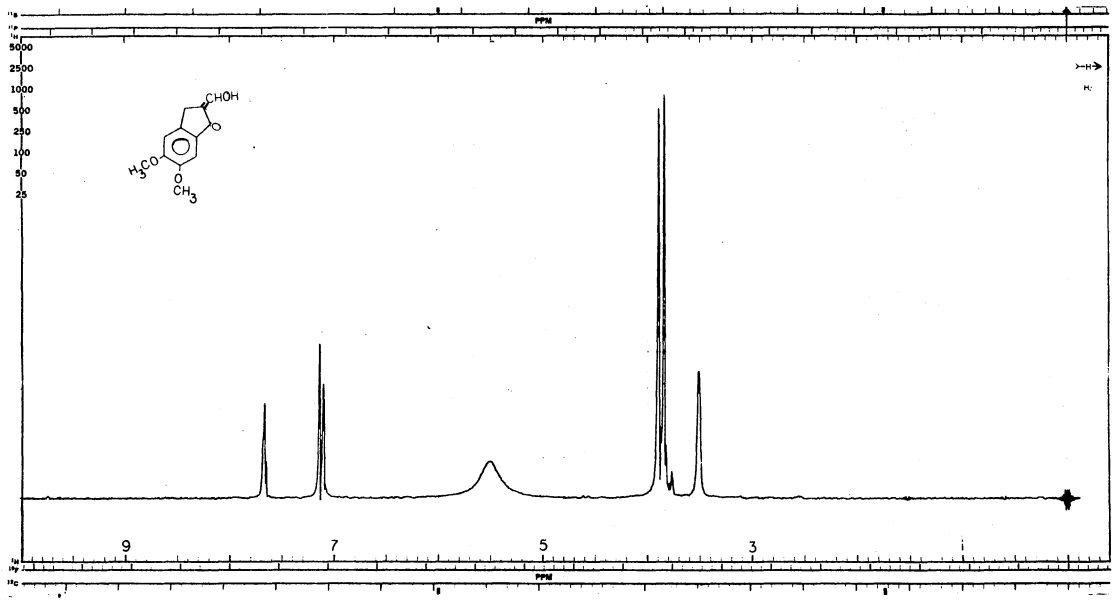
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 68 dB
S.W. . . .1000 Hz S.T. . . .500 Sec S.O. . . 83701 Hz S.A. . . . 5.0
Lock . . . . HOMO

PLATE XVI



XVIa. 5,6-Dimethoxy-1-indanone (134)

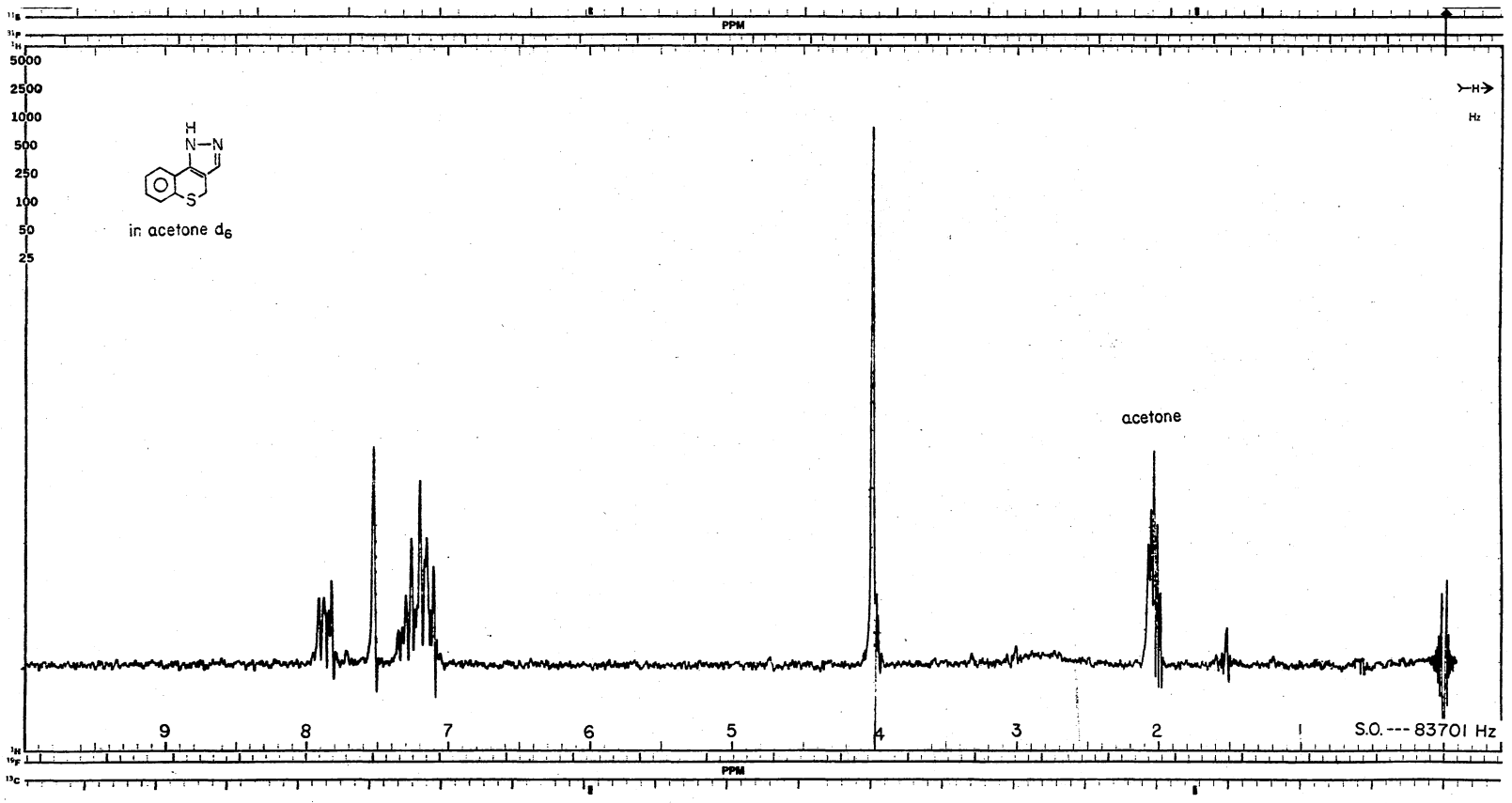
Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 55 dB
S.W. . . .1000 Hz S.T. . . .250 Sec S.O. . . 83701 Hz S.A. 4
Lock . . . . HOMO



XVIb. 2-(Hydroxymethylene)5,6-dimethoxy-1-indanone (98)

Solvent. . .$DCCl_3$ O.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 63 dB
S.W. . . .1000 Hz S.T. . . .500 Sec S.O. . . 83701 Hz S.A. . . .1.25
Lock . . . . HOMO

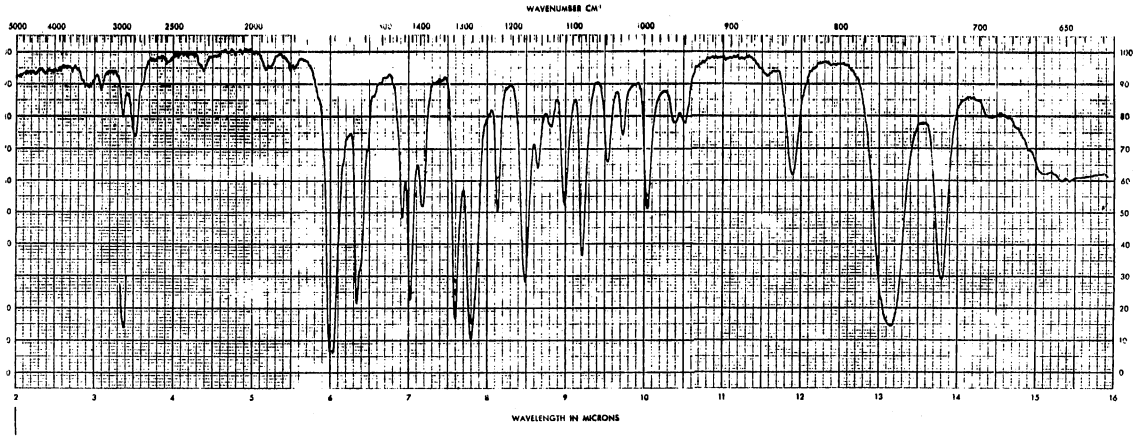
PLATE XVII



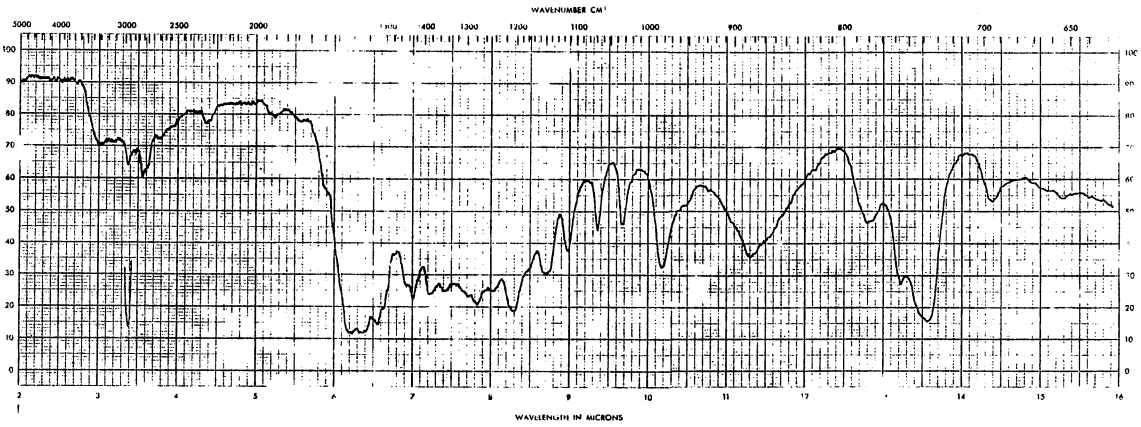
1,4-Dihydro[1]benzothiopyrano[4,3-c]pyrazole (65)

Solvent. . .$DCCl_3$ 0.F. . .100.1 MHz F.B. . . . . .2 R.F. . . 63 dB
S.W. . . .1000 Hz S.T. . . .500 Sec S.O. . . 83701 Hz S.A. . . . 6.3
Lock . . . . HOMO

PLATE XVIII

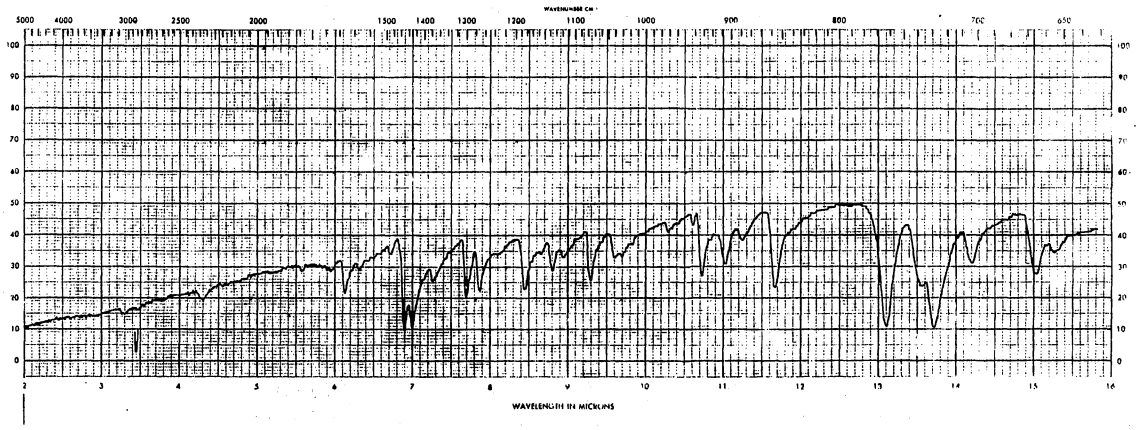


XVIIIa. Thiochroman-4-one (90)

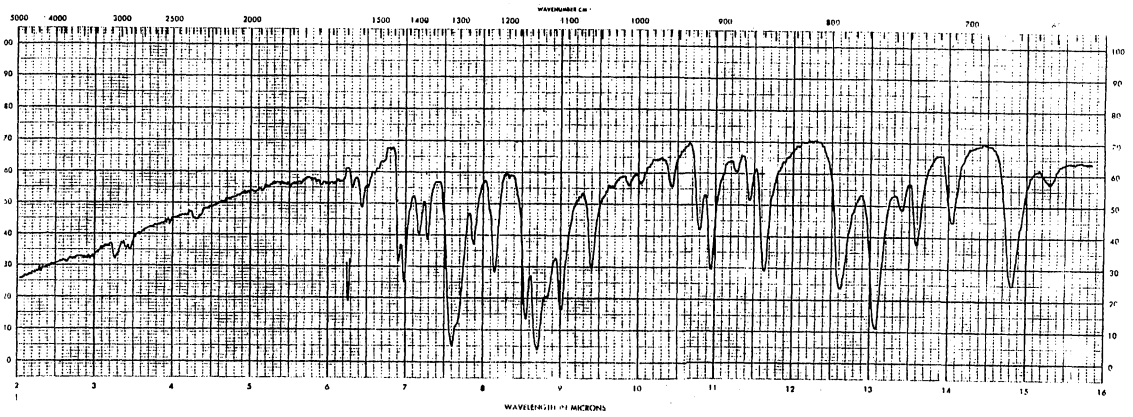


XVIIIb. 2-(Hydroxymethylene)thiochroman-4-one (94)

PLATE XIX

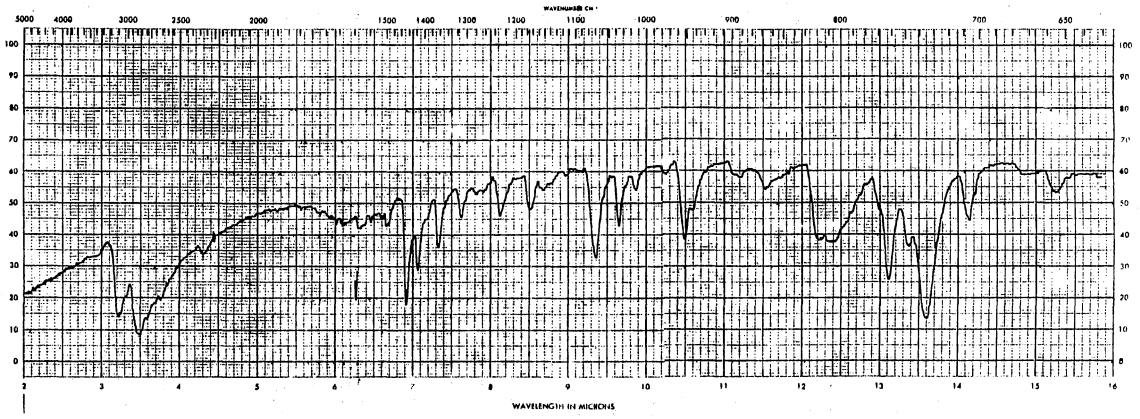


XIXa. 4H-[1]Benzothiopyrano[3,4-d]isoxazole (68)

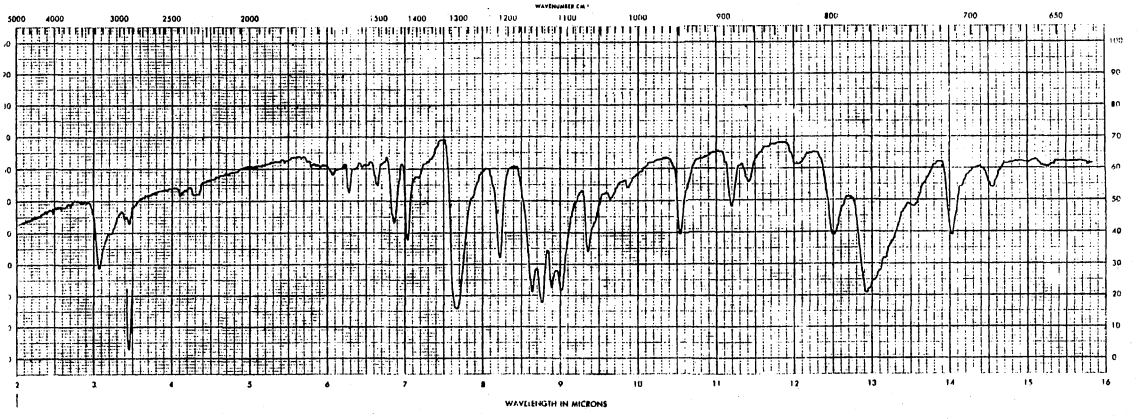


XIXb. 4H-[1]Benzothiopyrano[3,4-d]isoxazole 5,5-dioxide (72)

PLATE XX

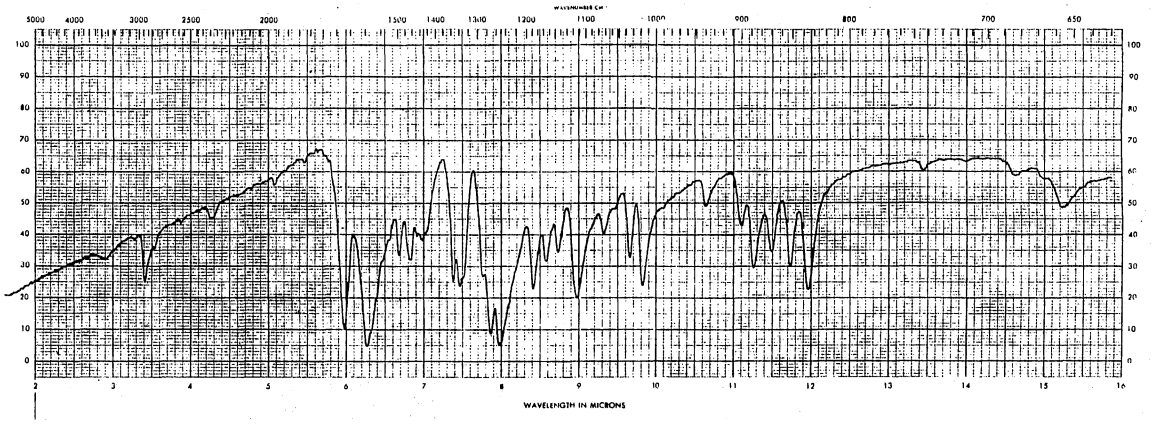


XXa. 1,4-Dihydro[1]benzothiopyrano[4,3-c]pyrazole (65)

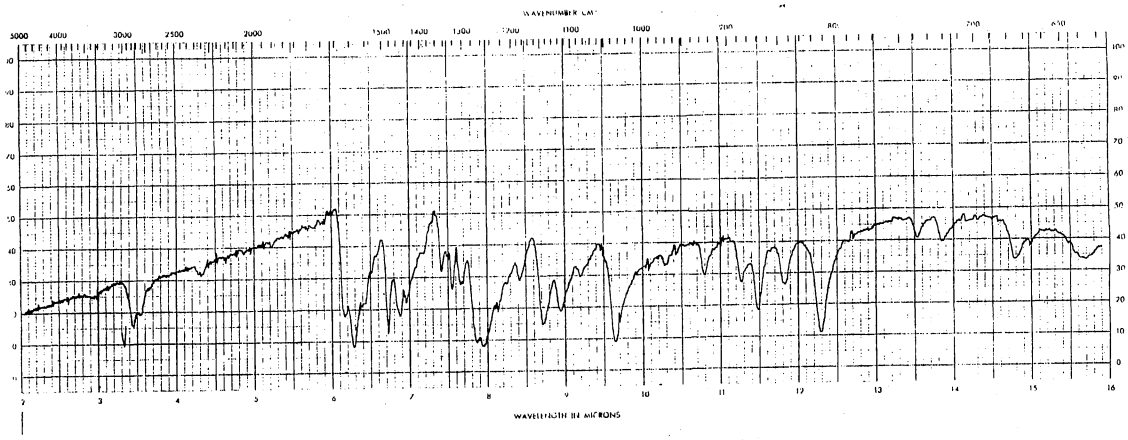


XXb. 1,4-Dihydro[1]benzothiopyrano[4,3-c]pyrazole 5,5-dioxide (70)

PLATE XXI

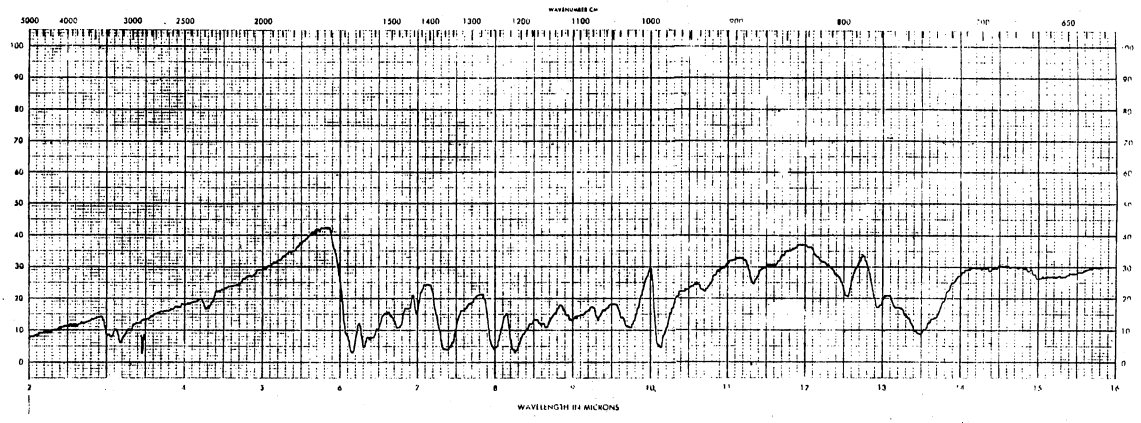


XXIa. 6-Methoxy-1-tetralone (89)

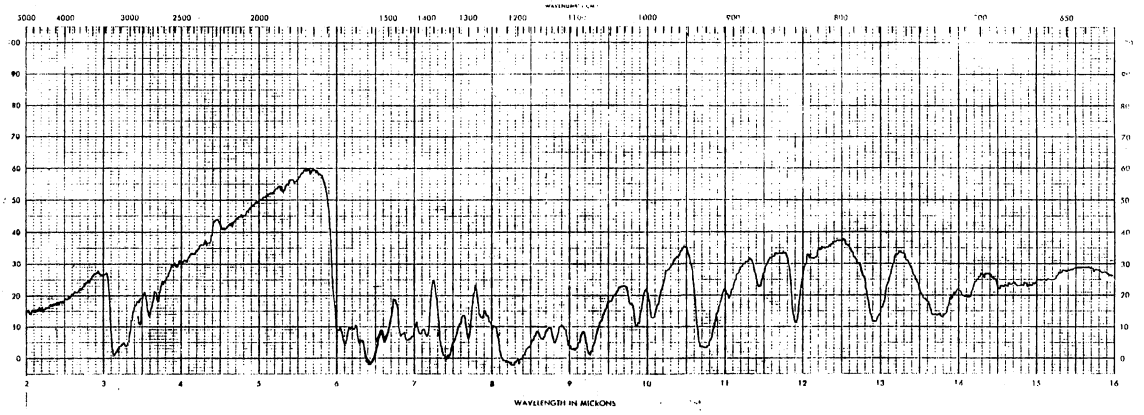


XXIb. N-Methyl-6-methoxy-1-tetralone imine (86)

PLATE XXII

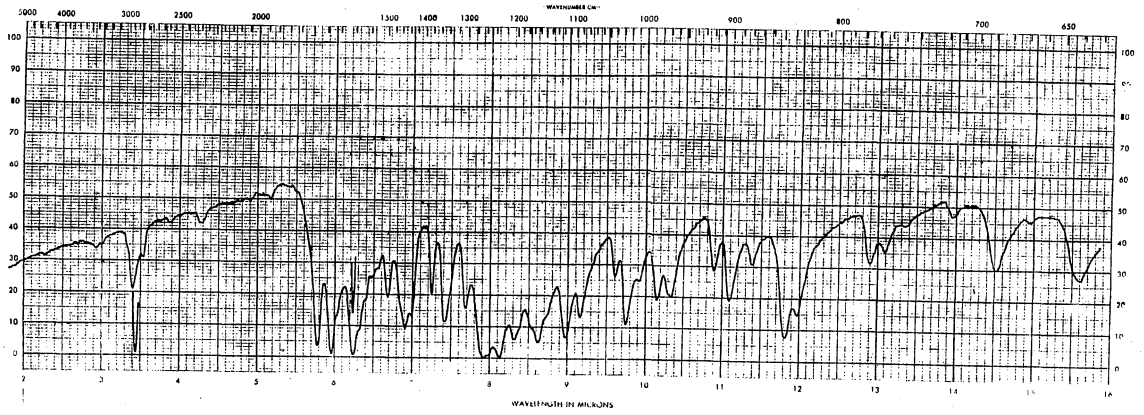


XXIIa. 1-[(4-Oxothiochroman-3-yl)methylene]-2-thiourea (78)

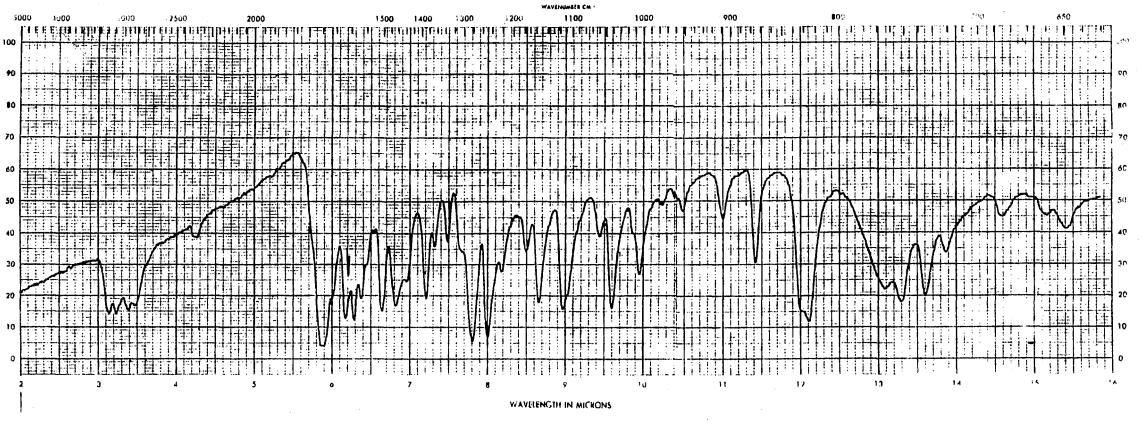


XXIIb. [(1,2,3,4-Tetrahydro-6-methoxy-1-oxo-2-naphthyl)-methylene]-2-thiourea (77)

PLATE XXIII

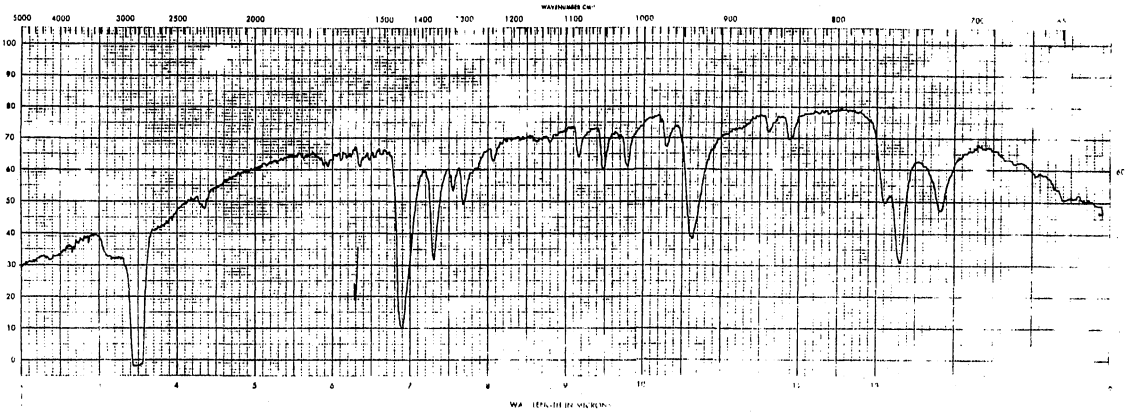


XXIIIa. Methyl 6-methoxy-2-methyl-1-oxo-1,2,3,4-tetrahydro-naphthalene-2-carboxylate (97)

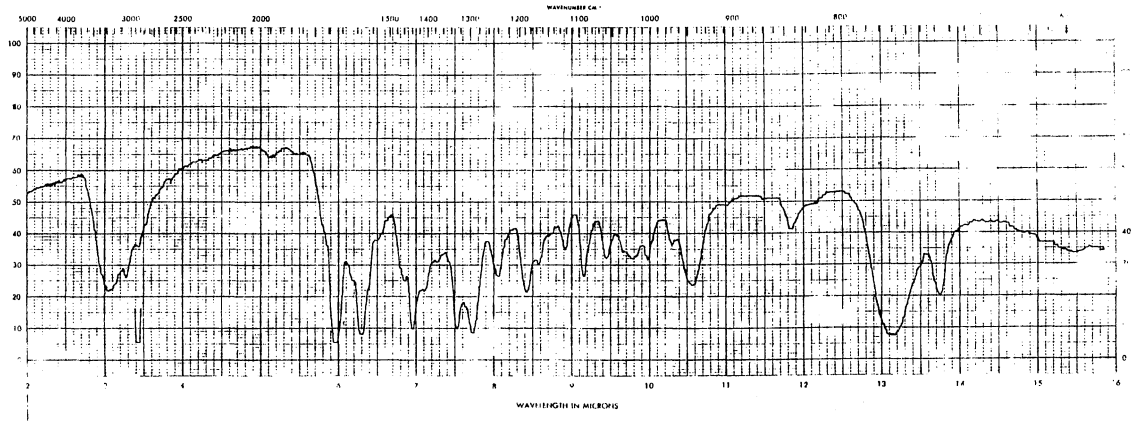


XXIIIb. 2,3a,4,5-Tetrahydro-3a-methyl-7-methoxy-3H-benz[g]-indazol-3-one (79)

PLATE XXIV

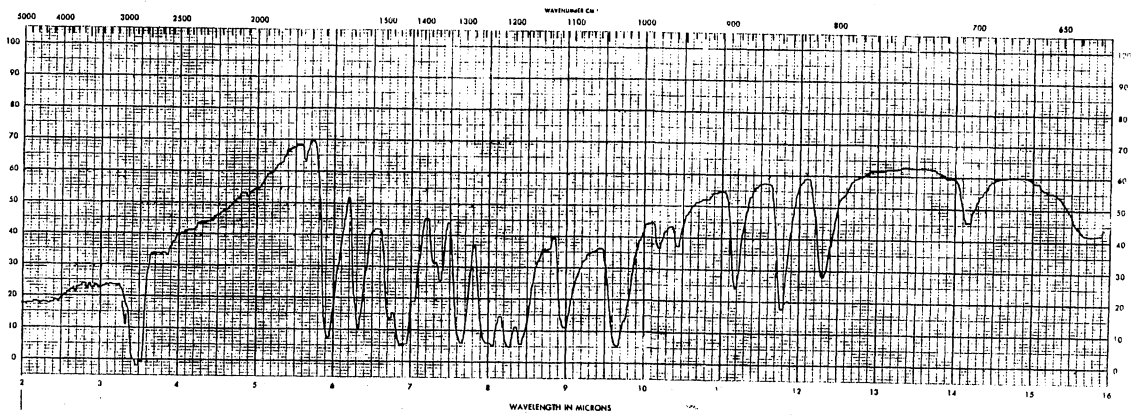


XXIVa. Thiochroman-4-one Oxime (74)

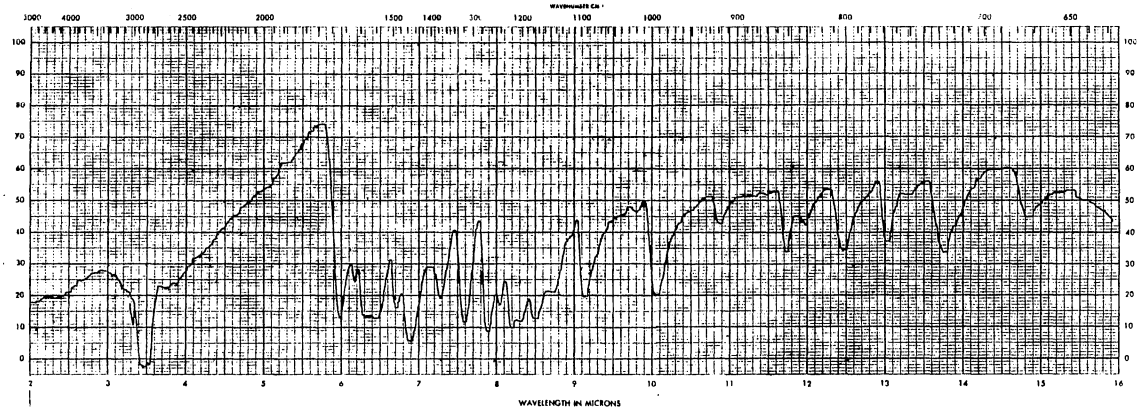


XXIVb. 3,4-Dihydro-1,4-benzothiazepin-5-(2H)-one (75)

PLATE XXV

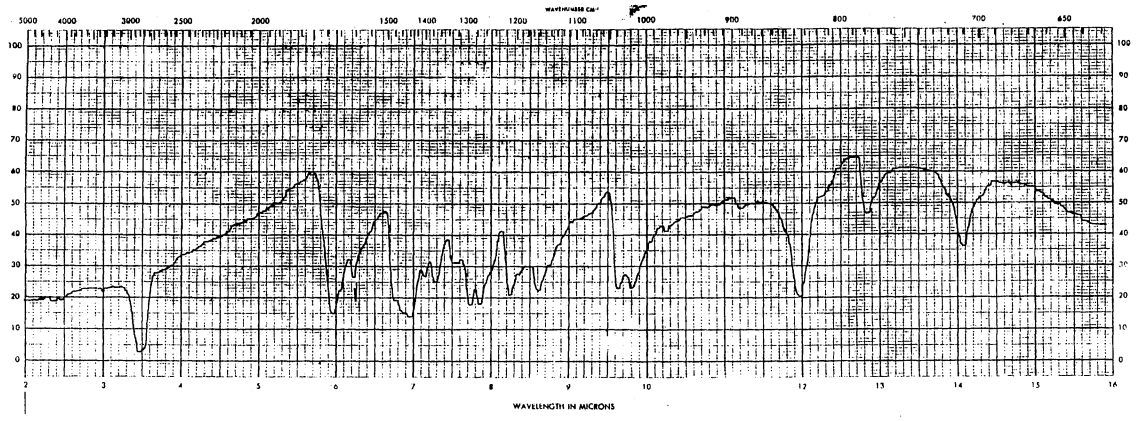


XXVa. 5,6-Dimethoxy-1-indanone (134)

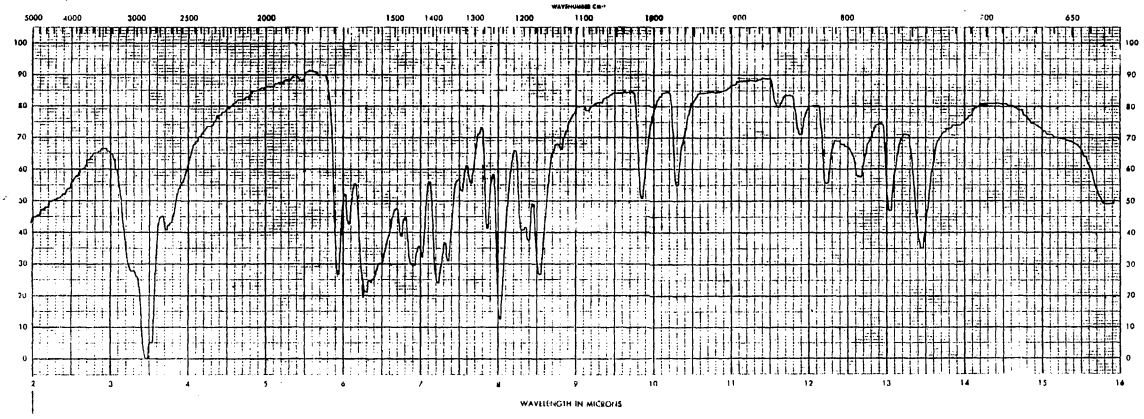


XXVb. 2-(Hydroxymethylene)5,6-dimethoxy-1-indanone (98)

PLATE XXVI

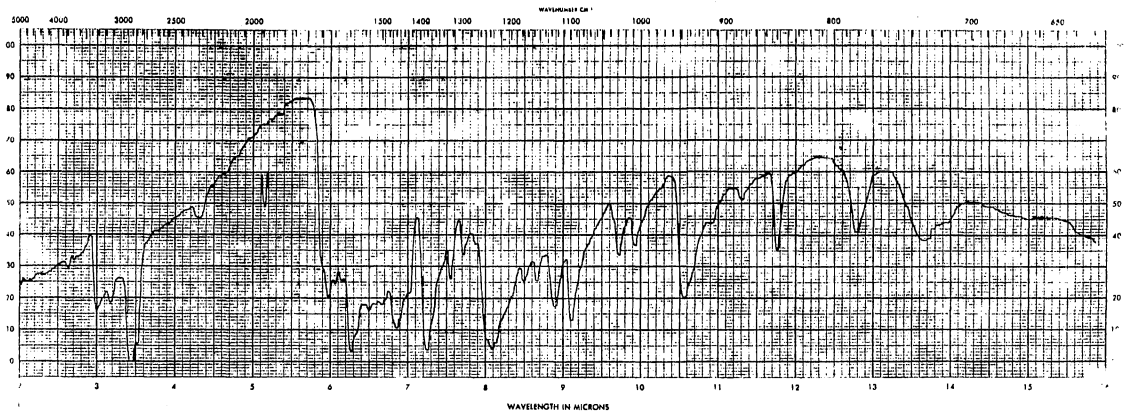


XXVIa. 6-Methoxy-1-indanone (95)

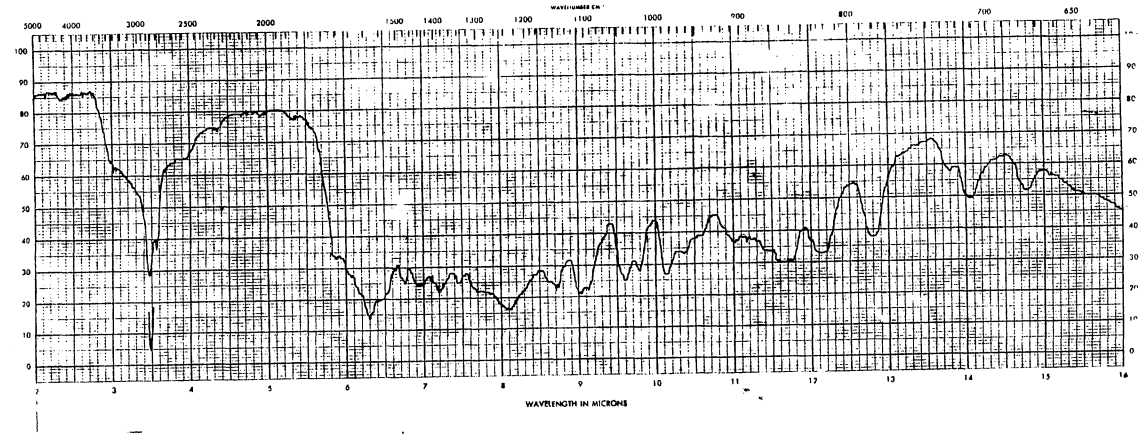


XXVIb. 2-(Hydroxymethylene)6-methoxy-1-indanone (95)

PLATE XXVII

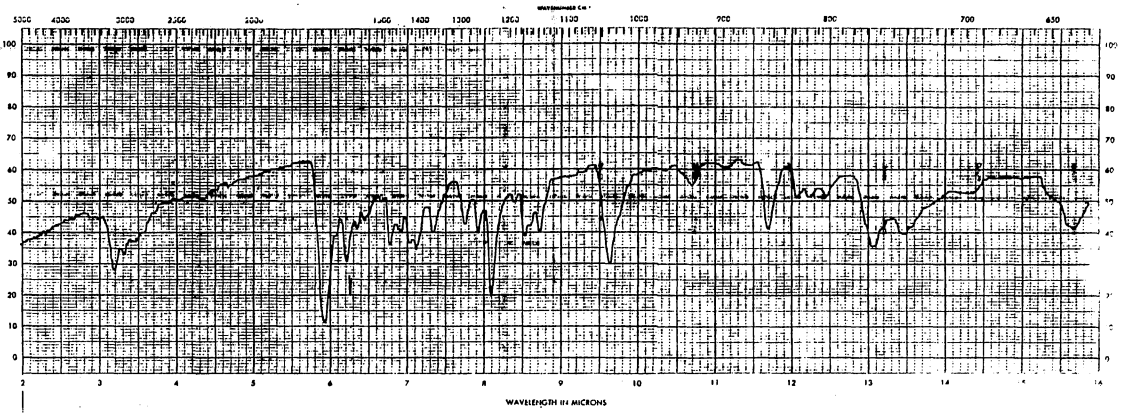


XXVIIa. [(1,2,3,4-Tetrahydro-6-methoxy-1-oxo-2-naphthyl)-methylene]urea (76)



XXVIIb. 2-(Hydroxymethylene)2-methoxybenzosuberone (96)

PLATE XXVIII



2,10,11,11a-Tetrahydro-7-methoxy-11a-methyl-1H-phenanthro-[1,2-c]pyrazol-1-one (83)

BIBLIOGRAPHY

1. Ainsworth, C., in "Organic Syntheses," Coll. Vol. IV (N. Rabjohn, Ed.), John Wiley and Sons, Inc., New York, 1963, p. 536.

2. Alauddin, M. and Martin-Smith, M., J. Pharm. Pharmacol., 14, 325 (1962).

3. Albert, A., "Heterocyclic Chemistry--An Introduction," 2nd edition, Oxford University Press, New York, 1968, Ch. 13.

4. Alberti, C. and Bregonzio, G., Farmaco (Pavia), Ed. Sci., 16, 540 and 557 (1961).

5. Alphen, V., Rec. Trav. Chim., 62, 485 (1943).

6. Anderson, D. M. V., Duncan, J. L., and Rossatti, F. J. C., J. Chem. Soc., 140, 4201 (1961).

7. Anderson, H. D. and Hammick, D. L., J. Chem. Soc., 1089 (1950).

8. Andrews, L. J. and Keefer, R. M., J. Amer. Chem. Soc., 71, 3644 (1949).

9. Andrews, L. J. and Keefer, R. M., J. Amer. Chem. Soc., 72, 3113 (1950).

10. Andrews, L. J. and Keefer, R. M., J. Amer, Chem. Soc., 72, 5034 (1950).

11. Andrews, L. J., Chem. Rev., 54, 713 (1954).

12. Andrews, L. J. and Keefer, R. M., J. Amer. Chem. Soc., 73, 4169 (1951).

13. Andrews, L. J. and Keefer, R. M., "Molecular Complexes in Organic Chemistry," Holden Day, San Francisco, 1964.

14. Andrews, L. J. and Keefer, R. M., Advan. Inorg. Chem. Radiochem., 3, 91 (1961).

15. Ansfield, F. J., Ramirez, G., Mackman, S., Bryan, C. J., and Curreri, A. R., Cancer Res., 29, 1062 (1969).

16. Arzamanova, I. G. and Gurjanova, E. N., Zh. Obshch. Khim., 36, 1157 (1966).

17. Auwers, K. v., Buschmann W., and Heiderreich, R., Ann., 435, 277 (1924).

18. Auwers, K. v. and Wolter, E., J. Prakt. Chem., 126, 204 (1930).

19. Bardou, L., Elguero, J., and Jacquier, R., Bull. Soc. Chem. France, 289, 297 (1967).

20. Beamann, A. G. and Duschinsky, R., U. S. Patent 3,317,352 (1967).

21. Becker, E. D., Liddel, U., and Shoolery, J. N., J. Mol. Spectry., 2, 1 (1958).

22. Behr, L., Fusco, R., and Jarbe, C. H., in "The Chemistry of Heterocyclic Compounds" (A. Weissberger, Ed.), Vol. 22, John Wiley and Sons, New York, N. Y., 1967.

23. Benesi, H. A. and Hildebrand, J. H., J. Amer. Chem. Soc., 70, 2832 (1948).

24. Benesi, H. A. and Hildebrand, J. H., J. Amer. Chem. Soc., 71, 2703 (1949).

25. Berens, K. and Shugar, D., Acta Biochim. Pol., 10, 25 (1963).

26. Berkeley, P. J. and Hanna, M. W., J. Phys. Chem., 67, 846 (1963).

27. Bolles, T. F. and Drago, R. S., J. Amer. Chem. Soc., 87, 5015 (1965).

28. Bolles, T. F. and Drago, R. S., J. Amer. Chem. Soc., 88, 3921 (1966).

29. Borello, E., Gazz. Chim. Ital., 89, 1437 (1959).

30. Boulton, A. J. and Katritzky, A. R., Tetrahedron, 12, 41 (1961); ibid., 12, 51 (1961).

31. Box, G. E. P. and Hunter, J. S., Technometrics, 3, 311 (1961).

32. Brady, L. W., Cancer 35, 76 (1975).

33. Brackman, W., Rec. Trav. Chim., 68, 147 (1949).

34. Brieglef, G., Pure. Appl. Chem., 4, 105 (1962).

35. Brown, H. C. and Brady, J. D., J. Amer. Chem. Soc., 74, 3570 (1952).

36. Brown, H. C. and Melchiore, J. J., J. Amer. Chem. Soc., 87, 5269 (1965).

37. Brown, J. P., Burgen, A. S. V., and Freeny, J., NMR Newsletter, 193, 24 (September 25, 1974).

38. Buchanan, G. W., Ross, D. A., and Stothers, J. B., J. Amer. Chem. Soc., 88, 4301 (1966).

39. Buchi, J. V., Meyer, H. R., Hirt, R., Hunziker, F., Eichenberger, E., and Lieberherr, R., Helv. Chim. Acta, 38, 670 (1955).

40. Buchta, E. and Buchholz, S., Ann., 688, 40 (1965).

41. Burger, A., ed., "Medicinal Chemistry," 3rd ed., John Wiley and Sons, Inc., New York (1970).

42. Burness, D. M., J. Org. Chem., 21, 97 (1956).

43. Bystrov, V. E., Granberg, I. I., and Sharova, G. I., Zh. Obshch. Khim., 35, 293 (1965).

44. Brande, E., Ann. Repts. Chem. Soc., 42, 105 (1945).

45. Bredereck, H., Effenberger, F., Botsch, H., and Rehn, H., Chem. Ber., 98, 1081 (1965).

46. Briegleb, G. and Kambeitz, J., Z. Physik. Chem., B32, 305 (1936).

47. Briegleb, G. and Czedkalla, Angew. Chem., 72, 401 (1960).

48. Briegleb, G., "Elektronen-Donor-Acceptor-Komplexe," Springer-Verlag, Berlin, 1961.

49. Briegleb, G., Z. Physik. Chem., B31, 58 (1936).

50. Califano, S., Piacenti, F., and Speroni, G., Spectrochim. Acta, 15, 86 (1959).

51. Carter, S. K., Cancer, 30, 1543 (1972).

52. Casoni, D. D., Magini, A., and Passerini, R., Gazz. Chim. Ital., 86, 797 (1956).

53. Caspi, E. and Piatak, D. M., Can. J. Chem., 41, 2294 (1963).

53a. Casy, A. F., "PMR Spectroscopy in Medicinal and Biological Chemistry," Academic Press, Inc., New York (1971).

54. Cauquis, G. and Basseleir, J. J., Ann. Chim. (France), 7, 745 (1962).

55. Champe, S. P. and Benzer, S., Proc. Natl. Acad. Sci., U. S., 48, 532 (1962).

56. Chaudhur, N. K., Montag, B. J., and Heidelberger, C., Cancer Res., 18, 318 (1958).

57. Chaudhuri, N. K., Mukherjee, K. L., and Heidelberger, C., Biochem. Pharmacol., 1, 328 (1959).

58. Chesnut, R. W., Durham, N. N., Mawdsley, E. A., Thyvelikakath, G. X., Berlin, K. D., and Fung, B. M., Biochem. J., 143, 789 (1974).

59. Chesnut, R. W., Ph.D. Dissertation, Oklahoma State University, Stillwater, Oklahoma, 1973.

60. Chesnut, R. W., Haslam, D. F., Durham, N. N., and Berlin, K. D., Can. J. Biochem., 50, 516 (1972).

61. Christensen, J. J., Eatough, D. J., and Izatt, R. M., Chem. Rev., 74, 351 (1974).

62. Claramount, R. M., Elguero, J., and Jacquier, R., Org. Magn. Resonance, 3, 595 (1971).

63. Clinton, R. O., et al., J. Org. Chem., 26, 279 (1961).

64. Clinton, R. O., Manson, A. J., Stonner, F. W., Neumann, H. C., Christiansen, R. G., Clarke, R. L., Ackerman, J. H., Page, D. F., Dean, J. W., Dickinson, W. B., and Carabateas, C., J. Amer. Chem. Soc., 83, 1478 (1961).

65. Cohen, J. L. and Connors, K. A., J. Pharm. Sci., 59, 1271 (1970).

66. Coleman, G., "Antibiotics," in "Data for Biochemical Research," edited by R. M. C. Dawson, D. C. Elliot, W. H. Elliot, and K. M. Jones, Oxford University Press, New York and Oxford, 1969.

67. Colter, A. K., Wang, S. S., Mergerle, G. H., and Ossip, P. S., J. Amer. Chem. Soc., 86, 3106 (1964).

68. Cooper, A. R., Crowne, C. W. P., and Farrell, P. G., Trans. Faraday Soc., 62, 2725 (1966).

69. Cooper, A. R., Crowne, C. W. P., and Farrell, P. G., Trans. Faraday Soc., 63, 447 (1967).

70. Cram, D. J. and Cran, J. M., Science, 183, 803 (1974).

71. Craven, B. M. and Gratland, G. L., J. Pharm. Sci., 59, 1666 (1970).

72. Curreri, A. R. and Ansfield, F. J., Cancer Chemotherapy Rep., 16, 478 (1962).

73. Curreri, A. R., Ansfield, F. J., Melver, F. A., Waisman, H. A., and Heidelberger, C., Cancer Res., 18, 474 (1958).

74. Cvetanovic, R. J., Duncan, F. J., and Falconer, W. E., Can. J. Chem., 42, 2410 (1964).

75. Cvetanovic, R. J., Duncan, F. J., Falconer, W. E., and Irwin, R. S., J. Amer. Chem. Soc., 87, 1827 (1965).

76. Cvetanovic, R. J., Duncan, F. J., Falconer, W. E., and Sunder, W. A., J. Amer. Chem. Soc., 88, 1602 (1966).

77. Dalling, D. K. and Grant, D. M., J. Amer. Chem. Soc., 89, 6612 (1967).

78. Davies, H. J. and Main, B. G., J. Chem. Soc., (C), 327 (1970).

79. Day, M. and Selbin, J., "Theoretical Inorganic Chemistry," Reinhold Press, New York, 1962, p. 83.

80. DeMaine, P. A. D., J. Chem. Phys., 26, 1042 (1957).

81. Devita, T. V. and Schein, P. S., N. Engl. J. Med., 288, 998 (1973).

82. DeVita, V. T., Serpick, A., and Carbone, P. P., Ann. Internal Med., 73, 881 (1970).

83. Dietrich, B., Lehn, J. M., and Simon, J., Angew. Chem., 86, 443 (1974).

84. Drago, R. S. and Rose, N. J., J. Amer. Chem. Soc., 81, 6141 (1959).

85. Duschinsky, R. and Gabriel, T. F., U. S. Patent 3,354,160 (1967).

86. Durham, N. N., Chesnut, R. W., Haslam, D. F., and Berlin, K. D., Ann. Okla. Acad. Sci., 77 (1974).

86a. Durham, N. N. and Keudell, K. C., Antimicrob. Ag. Chemother., 67, 72 (1969).

87. Durr, G. J., J. Med. Chem., 8, 253 (1965).

88. Duschinsky, R., U. S. Patent 3,185,690 (1965).

89. Duschinsky, R., Pleven, E., and Heidelberger, C., J. Amer. Chem. Soc., 79, 4559 (1957).

90. Elguero, J., Marzin, C., and Roberts, J. D., J. Org. Chem., 39, 357 (1974).

91. Ferguson, E. E., J. Chim. Phys., in press.

92. Finai, J. L. and Mooney, E. F., Spectrochim. Acta, 20, 1269 (1964).

93. Fisher, R. B. and Christie, G. A., "A Dictionary of Drugs," Schoken edition, 1972, New York.

94. Foster, R. and Fyee, ., Trans. Faraday Soc., 61, 1626 (1965).

95. Foster, R., Hammick, D. Lt., and Wardley, A. A., J. Chem. Soc., 3817 (1953).

96. Foster, R., Nature, 173, 222 (1954).

97. Fowden, L., Noe, F. F., Ridd, J. H., and White, R. F. M., Proc. Chem. Soc., 131 (1959).

98. Fravolini, A., Granolini, G., and Martani, A., Gazz. Chim. Ital., 103, 1073 (1973).

99. Finar, I. L. and Mooney, E., Spectrochim. Acta, 20, 1269 (1964).

100. Fischer, J. J. and Jardetzky, O., J. Amer. Chem. Soc., 87, 3237 (1965).

101. Gee, T. S., Yu, K. P., and Clarkson, B. D., Cancer, 23, 1019 (1969).

102. Gill, R. W., Progr. Med. Chem., 4, 39 (1965).

103. Gil-Av, A. and Herling, J., J. Phys. Chem., 66, 1208 (1962).

104. Gokel, G. W. and Cram, D. J., J. Chem. Soc., Chem. Comm., 481 (1973).

105. Grandberg, I. I., J. Gen. Chem. (USSR), 31, 2149, 2153 (1961); 33, 511 (1963).

106. Grandberg, I. I., Vasina, L. G., Volkova, A. S., and Kost, A. N., J. Gen. Chem. (USSR), 31, 1765 (1961).

107. Grant, D. M., "Magnetic Resonance," (C. K. Coogan et al., Eds.), Plenum Press, New York, 1970.

108. Grant, D. M. and Paul, E. G., J. Amer. Chem. Soc., 86, 2984 (1964).

109. Guarneri, M., Boll. Chim. Farm., 99, 259 (1960); Chem. Abstr., 54, 17798 (1960).

110. Guryanova, E. N., Russ. Chem. Rev., 37, 863 (1968).

111. Guthrie, R. W., Boris, A., Mullin, J. G., Mennona, F. A., and Kierstead, R. W., J. Med. Chem., 16, 257 (1973).

112. Guthrie, R. W., Boris, A., Mullin, J. G., Mennona, F. A., and Kierstead, R. W., J. Med. Chem., 16, 259 (1973).

113. Habraken, C. L. and Moore, J. A., J. Org. Chem., 30, 1892 (1965).

114. Hammes, G. G. and Tallman, D. E., Biochim. Biophys. Acta, 233, 17 (1971).

115. Hanna, M. W., Trigg, W., and Ashbaugh, A. L., unpublished results.

116. Hanna, M. W. and Ashbaugh, A. L., J. Phys. Chem., 68 (4), 811 (1964).

117. Hanna, M. W. and Ashbaugh, A. L., J. Phys. Chem., 68, 811 (1964).

118. Hashem, M. M., Ph.D. Dissertation, Oklahoma State University, Stillwater, Oklahoma, 1975.

119. Haslam, D. F., Ph.D. Dissertation, Oklahoma State University, Stillwater, Oklahoma, 1973.

120. Hatton, J. V. and Richards, R. E., Mol. Phys., 5, 153 (1962).

121. Hatton, J. V. and Richards, R. E., Mol. Phys., 3, 253 (1960).

122. Heath, D. F., "Organicphosphorus Poisons," Pergamon Press, Oxford, 1961.

123. Heidelberger, C. and Ansfield, F. J., Cancer Res., 23, 1226 (1963).

124. Heidelberger, C., Chaudhuri, N. K., Danneberg, P., Mooren, D., Griesbach, L., Duschinsky, R., Schnitzer, R. J., Pleven, E., and Scheiner, J., Nature, 179, 663 (1957).

125. Heidelberger, C. and Duschinsky, R., U. S. Patent 2,885,396 (1959).

126. Heidelberger, C., Leibman, K. C., Harbers, E., and Bhargava, P., Cancer Res., 17, 399 (1957).

127. Heidelberger, C., Progr. Nucleic Acid Res. Mol. Biol., 4, 1, (1965).

128. Helgeson, R. C., Koga, K., Timko, J. M., and Cram, D. J., J. Amer. Chem. Soc., 95, 3021 (1973).

129. Helgeson, R. C., Timko, J. M., and Cram. D. J., J. Amer. Chem. Soc., 95, 3023 (1973).

130. Helgeson, R. C., Timko, J. M., Moreau, P., Peacock, S. C., Mayer, J. M., and Cram, D. J., J. Amer. Chem. Soc., 96, 6762 (1974).

131. Henderson, E. S., Seminars Hematol., 6, 271 (1969).

132. Higuchi, T. and Kristiansen, H., J. Pharm. Sci., 59, 1601 (1970).

133. Hoffer, M., Duschinsky, R., Fox, J. J., and Young, N., J. Amer. Chem. Soc., 81, 4112 (1959).

134. Hoffer, M., U. S. Patent 3041, 335 (1962).

135. Huttel, R., Franke, K., Martin, H., and Reidel, J., Chem. Ber., 93, 1433 (1960).

136. Huggins, C. M., Pimentel, G. C., and Schoolery, J. N., J. Chem. Phys., 23, 1244 (1955).

137. Huttel, R., Reidel, J., Martin, H., and Franke, K., Chem. Ber., 93, 1425 (1960).

138. Jacquier, R., Petrus, C., Petrus, F., and Valeutin, M., Bull. Soc., Chim. France, 3672, 2678 (1970).

139. Jautelat, M. and Roberts, J. D., unpublished results.

140. Jawtez, E. and Gunnison, J. B., Antibiot. Chemotherap., 2, 234 (1952).

141. Johnson, W. S. and Shelberg, W. E., J. Amer. Chem. Soc., 67, 1745 (1945), 69, 2942 (1947).

142. Johnson, W., Anderson, J., and Shelberg, W., J. Amer. Chem. Soc., 66, 218 (1944).

143. Johnson, W., Petersen, J., and Gutsche, C., J. Amer. Chem. Soc., 69, 2942 (1947).

144. Kagarise, R. E., Spectrochim. Acta, 19, 629 (1963).

145. Kakemi, K., Sezaki, H., Mitsunga, T., and Nakano, M., J. Pharm. Sci., 59, 1597 (1970).

146. Kasturi, T. R. and Arunachalam, T., Indian J. Chem., 8, 203 (1970).

147. Katritsky, A. R. and Boulton, A. J., Spectrochim. Acta, 17, 238 (1961); Tetrahedron, 21, 1693 (1965).

148. Katritsky, A. R., Quart. Revs. (London), 13, 353 (1959).

149. Katritzky, A. R., Maine F. W., and Golding, S., Tetrahedron, 21, 1693 (1965).

150. Katritzky, A. R. and Maine, F. W., Tetrahedron, 20, 229, 315 (1964).

151. Kent, R. J., Jhwaja, T. A., and Heidelberger, C., J. Med. Chem., 13, 70 (1970).

152. Ketelaar, J. A. A., van de Stolpe, C., Goudsmit, A., and Dzcubas, W., Rec. Trav. Chim., 71, 1104 (1952).

153. Ketelaar, J. A. A., Chem. Wekblad, 52, 218 (1956).

154. King, R. B. and Heekley, P. R., J. Amer. Chem. Soc., 96, 3118 (1974).

155. Knorr, L., Ber., 16, 2597 (1883); ibid., 17, 546 (1884).

156. Koll, W. and Fleischmann, G., Arch. Exptl. Path. Pharmakol., 198, 390 (1941); Chem. Abstr., 39, 3355 (1945).

157. Kosower, E. M., Prog. Phys. Org. Chem., 3, 81 (1965).

158. Kosower, E. M., "Molecular Biochemistry," McGraw Hill, New York, 1962, pp. 185-195.

159. Kost, A. N. and Grandberg, I. I., Advan. Heterocycl. Chem., 6, 347, 429 (1966).

160. Kristiansen, H., Nakano, M., Nakano, N. I., and Higuche, T., J. Pharm. Sci., 59, 1103 (1970).

161. Kuboyama, A., J. Amer. Chem. Soc., 86, 164 (1964).

162. Kyba, E. P., Koga, K., Sousa, L. R., Siegel, M. G., and Cram, D. J., J. Amer. Chem. Soc., 95, 2692 (1973).

163. Kyba, E. P., Siegel, M. G., Sousa, L. R., Sogah, G. D., and Cram, D. J., J. Amer. Chem. Soc., 95, 2691 (1973).

164. Lardy, H., Federation Proc., 27, 1278 (1968).

165. Laszalo, P. and Williams, D. H., J. Amer. Chem. Soc., 88, 2799 (1966).

166. Lauransan, J., Pineau, P., and Lascombe, J., J. Chim. Phys., 63, 635 (1966).

167. Lehninger, A. L., "Biochemistry," Worth Publishers, New York, 1970.

168. Lematre, J. and Soulier, J., C. R. Acad. Sc. Paris, 265 (c), 199 (1967).

169. Lematre, J. and Soulier, J., C. R. Acad. Sci., Paris, Ser. C, 265, 199 (1967); Chem. Abstr., 68, 3062 (1968).

169a. Levy, G. C. and Nelson, G. L., "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," John Wiley and Sons, Inc., New York, 1972.

170. Liptay, W., Z. Electrochem., 65, 375 (1961).

171. Livingston, R. B. and Carter, S. K., "Single Agents in Cancer Chemotherapy," Plenum Press, London, 1970.

172. Lowdin, P. O., Adv. Quantum. Chem., 2, 213 (1965); Pullman, B. and Pullman, A., "Quantum Biochemistry," Interscience, New York, 1963.

173. Manson, A. J. and co-workers, J. Med. Chem., 6, 1 (1963).

174. Manson, A. J., Stommer, F. W., Beyler, A. L., Potts, G. O., and Arnold, A. J., J. Amer. Chem. Soc., 81, 1513 (1959).

175. Marchetti, E. and Donini, P., Gazz. Chim. Ital., 86, 1133 (1961).

176. Martin-Smith, M. and Sugrue, F., J. Pharm. Pharmacol., 16, 568 (1964).

177. Maugh, T. H., Science, 184, 147 (1974).

178. Maw, G. A., "Sulfur in Organic and Inorganic Chemistry," (A. Semming, ed.), Vol. 2, Marcel Dekker, Inc., New York, 1972, Chap. 15.

179. McConnell, H. M., J. Chem. Phys., 28, 430 (1958).

180. McGlynn, S. P., Chem. Rev., 58, 1113 (1958).

181. Meites, L., ed., "Handbook of Analytical Chemistry," McGraw-Hill, New York, 1963.

182. Menger, F. M. and Bender, M. L., J. Amer. Chem. Soc., 88, 131 (1966).

183. Meyer, W. L., Clemans, G. B., and Huffman, R. W., Tetrahedron Lett, 4255 (1966).

184. Michel, G. and Duyckaerts, G., Spectrochim. Acta, 21, 279 (1965).

185. Mirone, P. and Vampiri, M., Atti. Accad. Mazl. Lincei, 12, 583 (1952); Chem. Abstr., 46, 9423 (1952).

186. Moore, J. A. and Habraken, C. L., J. Amer. Chem. Soc., 86, 1456 (1964).

187. Moore, T. S., Shepherd, F. and Goodall, F., J. Chem. Soc., 1447 (1931).

188. Morcillo, J. and Gallego, E., Anales de la Real Soc. Espan. de Fis. y Quim., 56B, 263 (1960).

189. Moreau, W. M. and Weiss, K., J. Amer. Chem. Soc., 88, 204 (1966).

190. Moreau, W. M. and Weiss, K., "Charge Transfer Properties of Sulfur Compounds," Mechanisms of Reactions of Sulfur Compounds, ed., Kharasch, Thyagarajan, and Khodaiv, Vol. 2 (1968).

191. Morgan, J. G., Berlin, K. D., Durham, N. N., and Chesnut, R. W., J. Heterocycl. Chem., 8, 61 (1971).

192. Muhs, M. A. and Weiss, F. T., J. Amer. Chem. Soc., 84, 4697 (1962).

193. Muskherjee, K. L. and Heidelberger, C., J. Biol. Chem., 235, 433 (1960).

194. Mulliken, R. S., J. Amer. Chem. Soc., 74, 811 (1952).

195. Mulliken, R. S., J. Chem. Phys., 61, 20 (1964).

196. Mulliken, R. S. and Person, W. B., Ann. Rev. Phys. Chem., 13, 107 (1962).

197. Mulliken, R. S., J. Amer. Chem. Soc., 74, 811 (1952).

198. Murrell, J. N., Quart. Rev., 15, 191 (1961).

199. Nakamoto, K., J. Amer. Chem. Soc., 74, 1739 (1952).

200. Nash, C. P., J. Phys. Chem., 64, 950 (1960).

201. National Cancer Institute Act, Public Law 75-244, 75th Congress, Senate 2067, August 5, 1937.

202. Niedzielski, R. J., Drago, R. S., and Middaugh, R. L., J. Amer. Chem. Soc., 86, 1694 (1964).

203. Northey, E. H., "The Sulfonamides and Allied Compounds," Reinhold, New York, N. Y., 1948.

204. Parini, V. P., Uspekhi Khim., 31, 882 (1962).

205. Parini, V. P., Russ. Chem. Rev., 31, 408 (1962).

206. Paquette, L. A., "Principles of Modern Heterocyclic Chemistry," W. A. Benjamine, Inc., New York, 1968, Chapter 6.

207. Pedersen, C. J. and Frensdorff, H. K., Angew. Chem. Internat. Edit. Engl., 11, 16 (1972).

207a. Pederson, R. L., Johnson, J. L., Holysz, R. P., and Ott, A. C., J. Amer. Chem. Soc., 79, 1115 (1957).

208. Peerson, W. B. and Mulliken, R. S., Molecular Complexes: A Lecture and Reprint Volume, Wiley, New York (to be published).

209. Peover, M. E., Proc. Chem. Soc., 167 (1963).

210. Peover, M. E., Trans. Faraday Soc., 60, 417 (1964).

211. Perkins, ., Biochem. J., 111, 195 (1969).

212. Person, W. B., J. Amer. Chem. Soc., 87, 167 (1965).

213. Philips, J. P., "Spectra-Structure Correlation," Academic Press, New York, 1964, Ch. 3.

214. Platt, J. R., Ann. Rev. Phys. Chem., 10, 367 (1959).

215. Pollak, R. D. and Rosenkranz, H. S., Cancer Res., 27, 1214 (1967).

216. Popov, A. I., Humphrey, R. E., and Person, W. B., J. Amer. Chem. Soc., 82, 1850 (1960).

217. Price, W. C., Ann. Rev. Phys. Chem., 11, 140 (1960).

218. Prystas, M. and Sorm, F., Collect. Czech. Chem. Commun., 30, 1900 (1965).

219. Purser, J. M. and Spielvogel, J. M., Inorg. Chem., 7, 2156 (1968).

220. Raiziss, G. W., Clemence, L. W., and Freifelder, M., J. Amer. Chem. Soc., 63, 2739 (1941).

221. Rao, C. N. R., "Ultraviolet and Visible Spectroscopy," 2nd ed., Plenum Press, New York, 1967.

222. Refn, S., Spectrochim. Acta, 17, 40 (1961).

223. Reitemeier, R. J., Moertel, C. G., and Hahn, R. G., Cancer Research, 30, 1425 (1971).

224. Roberts, J. D. and Grutzner, J. B., unpublished results on methyl substituted norbornane derivatives.

224a. Romo, J. and Vivar, R. D., J. Amer. Chem. Soc., 79, 1118 (1957).

225. Ronayne, J. and Williams, D. H., Chem. Comm., 712 (1966).

226. Rose, N. J. and Drago, R. S., J. Amer. Chem. Soc., 81, 6138 (1959).

227. Rosen, B., Rothman, F., and Weigert, M. G., J. Mol. Biol., 44, 363 (1969).

228. Ross, S. D. and Kuntz, I., J. Amer. Chem. Soc., 76, 3000 (1954).

229. Ruzicka, L. and Plattner, P. A., Helv. Acta, 121, 1717 (1938).

230. Sam, J. D. and Simmons, H. E., J. Amer. Chem. Soc., 94, 4024 (1972).

231. Scuri, R., Boll. chim. farm., 109, 674 (1970), Chem. Abstr., 75, 3806 (1971); Scuri R., Farmaco, Ed. Prat., 25, 588, 580 (1970), Chem. Abstr., 73, 118918 (1970); Scuri, R., Faini, D., and Venezini, S., Boll. chim. farm., 108, 706 (1969); Chem. Abstr., 72, 111352 (1969).

232. Silverstein, R. M. and Bassler, G. C., "Spectrometric Identification of Organic Compounds," 2nd ed., John Wiley and Sons, New York, 1967, Ch. 5.

232a. Smith, R. F., Shay, D. E., and Doorenbos, N. J., J. Pharm. Sci., 53, 1214 (1964); Smith, R. F., Shay, D. E., and Doorenbos, N. J., Appl. Microbiol., 11, 542 (1963); Smith, R. F., Shay, D. E., and Doorenbos, N. J., J. Bacteriol., 85, 1295 (1963); and Shay, D. E., Smith, R. F., and Doorenbos, N. J., Proc. Penn. Acad. Sci., 31, 113 (1962).

233. Sousa, L. R., Hoffman, D. H., Kaplan, L., and Cram, D. J., J. Amer. Chem. Soc., 96, 7100 (1974).

233a. Stothers, J. B., "Carbon-13 NMR Spectroscopy," Academic Press, New York, 1972.

234. Szent-Gyorgyi, A., Introduction to a Submolecular Biology, Academic Press, 1960.

235. Taft, R. W. and Carten, J. W., J. Amer. Chem. Soc., 86, 4199 (1964).

236. Taylor, E., McKillop, A., Shvo, Y., and Hawks, G., Tetrahedron, 23, 2081 (1967).

237. Teeney, J., Newman, G. A., and Pauwels, P. J. S., J. Chem. Soc., (C), 1842 (1970).

238. Terpinski, J. and Kozluk, T., Rocz. Chem., 48, 29 (1974); Chem. Abstr., 80, 145526n.

239. Thyvelikakath, G. X., Bremlet, L. J., Snider, T. E., Morris, D. L., Haslam, D. F., White, W. D., Purdie, N., Durham, N. N., and Berlin, K. D., J. Heterocycl. Chem., 11, 189 (1974).

240. Timko, J. M., Helgeson, R. C., Newcomb, M., Gokel, G. W., and Cram, D. J., J. Amer. Chem. Soc., 96, 7097 (1974).

241. Ting-Po, I. and Grunwald, H., J. Amer. Chem. Soc., 96, 2879 (1974).

242. Undheim, K. and Gacek, M., Acta Chem. Scand., 23, 294 (1969).

243. Vinogradov, S. N. and Linnell, R. H., "Hydrogen Bonding," Van Nostrand Reinhold Company, New York, 1971.

243a. Vogtle, F. and Neuman, P., Synthesis., 85 (1973).

244. Yamada, H. and Kozima, K., J. Amer. Chem. Soc., 82, 1543 (1960).

245. Young, N. C., Burchenal, J. H., Fecher, R., Duschinsky, R., and Fox, J. J., J. Amer. Chem. Soc., 83, 4060 (1961).

246. Watanabe, K. A. and Fox, J. J., J. Heterocycl. Chem., 6, 109 (1969).

247. Wierzchowski, K. L., Litonska, E., and Shugar, D., J. Amer. Chem. Soc., 87, 4621 (1965).

248. Wiley, R. H. and Jussung, K. F., J. Amer. Chem. Soc., 79, 4395 (1957).

249. Williams, J. K., J. Org. Chem., 29, 625 (1964).

250. Woodson, B. and Joklik, W. K., Proc. Natl. Acad. Sci. U. S., 54, 946 (1965).

251. Zderic, J. A. and co-workers, Chem. Ind. (London), 1625 (1960).

252. Zubrod, G. C., "New Ideas in Cancer Chemotherapy," Intrascience Symposium, Santa Monica, 1973.

VITA

George Xavier Thyvelikakath

Candidate for the Degree of

Doctor of Philosophy

Thesis: SYNTHESIS OF SELECTED HETEROCYCLES AND SPECTRAL ANALYSIS OF MOLECULAR COMPLEXES BETWEEN HETEROCYCLIC STEROIDAL SYSTEMS (AND MODEL COMPOUNDS) AND ANTICANCER AGENTS

Major Field: Chemistry

Biographical:

Personal Data: The author was born in Cochin, Kerala state, India, on April 29, 1943, the son of Xavier and Annie Thyvelikakath. On August 26, 1974 he was married to Mary Devassy Manikkil.

Education: The author graduated from Saint Peter's High School in Kumbalanghy, Cochin, Kerala state, India in 1960. He received the Bachelor of Science degree with a major in Chemistry and minors in Physics and Mathematics from University of Kerala, Kerala, India, in April, 1965 and a Master of Science degree from the University of Southwestern Louisiana, Lafayette, Louisiana, in May, 1972. In July, 1975 he completed the requirements for the Doctor of Philosophy degree at Oklahoma State University, Stillwater, Oklahoma, USA.

Professional Experience: The author served as chairman of student body council at Saint Peter's High School, 1959-60, and received the Alexander Parambithara Memorial prize for the outstanding student achievements in 1960. He was employed by Saint Peter's High School and Jaya Tutorial College in Kumbalanghy, 1965-67. From September, 1969 to May, 1971, he was a graduate teaching assistant at the University of Southwestern Louisiana. He was a graduate teaching and research assistant (supported by the National Cancer Institute) in the Department of Chemistry at Oklahoma State University, 1972-75. The author was recipient of summer research fellowships (a Dow Fellowship in 1974 and a Gulf Fellowship in 1975. He received the Outstanding Graduate Assistant Teacher Award for 1974 at Oklahoma State University.

Membership in Professional Societies: The author is a member of American Chemical Society, Sigma Xi, and Phi Lambda Upsilon and a Fellow of Indian Chemical Society.