12-1961

Some Reactions of Phenothiazine and Certain Benzophenothiazines

John Charles Gilmer
University of Tennessee - Knoxville

Recommended Citation

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<div align="right">David A. Shirley, Major Professor</div>

We have read this dissertation and recommend its acceptance:

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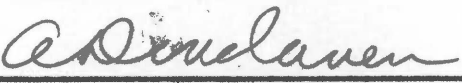(Original signatures are on file with official student records.)

December 4, 1961

To the Graduate Council:

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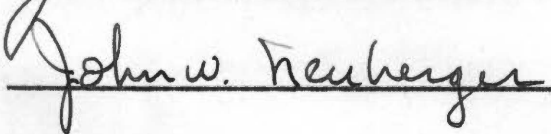
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Major Professor

We have read this dissertation and recommend its acceptance:

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Accepted for the Council:

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Dean of the Graduate School

SOME REACTIONS OF PHENOTHIAZINE AND CERTAIN BENZOPHENOTHIAZINES

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

John Charles Gilmer

December 1961

TO MITZI

ACKNOWLEDGEMENT

TABLE OF CONTENTS

IV. (Continued)

LIST OF TABLES

CHAPTER I

INTRODUCTION

Cancer chemotherapy has been a field of much interest and activity for the past 15 years. The impetus to the search for an effective chemical agent is the worldwide mortality rate from cancer, placed by estimates at more than two million people annually.

Surgery and radiation are the best methods for the treatment of localized cancers. However, these techniques cannot be used effectively against scattered cancers or cancers of the blood-making organs. There is at present a great effort underway to find chemical agents which can be used against the latter types of cancer.

Random screening of materials for anticancer activity is the responsibility of the Cancer Chemotherapy National Service Center (CCNSC). Each compound submitted to the CCNSC is tested for activity against 3 transplanted animal tumors in mice: solid subcutaneous forms of sarcoma 180 and adenocarcinoma 755, and the ascitic form of leukemia L1210. In addition the alkylating agents, compounds able to replace hydrogen with alkyl groups in biologically important materials, are subjected to tests on the Walker 256 carcinoma which is sensitive to these agents.

In 1959 it was estimated[1] that chemical substances were moving through the cancer screening program at the rate of 50,000 per year. Exact figures are not available on the total number of substances which have been tested but the number for the 15-year period before 1960

1

probably was greater than 300,000. Crude beers provided by the anti-
biotic fermentation programs of industry comprise the larger portion
of these chemicals. The number of specific chemical compounds sub-
mitted for testing had reached 61,000 in July, 1961. Only 167 compounds
were placed in clinical trial between 1940 and 1960 and of these, 20
have proved to be clinically useful.

It must be emphasized that at present most of the cancer chemo-
therapeutic drugs considered to be clinically useful produce only
temporary remission of one or more of the forms of cancer, prolonging
the life of the patient. However, there have been a few reports of
excellent results brought about by chemotherapy. In 1958 Hertz,
Bergenstal, Lipsett, Price, and Hilbish[2] observed complete remission
of choriocarcinoma in 5 patients for periods from 8 to 29 months
through the administration of methotrexate, N-{$\underline{p}$-{[(2,4-diamino-6-
pteridinyl)methyl]methylamino}benzoyl}glutamic acid (I-1). Recently
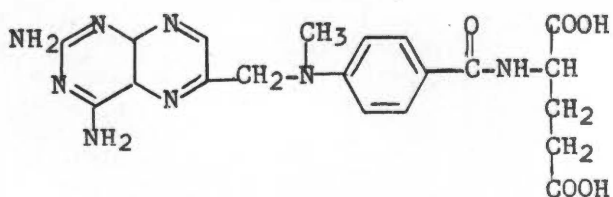
I-1

Hertz, Lewis, and Lipsett[3] reported that of 63 women treated for chorio-
carcinoma with methotrexate, 30 had been free of disease for periods
from 3 months to 5 years.

The group of compounds known as alkylating agents constitutes
the majority of the effective cancer chemotherapeutic drugs. These
compounds are separated into three classes, nitrogen mustards (I-2),

ethylenimines (I-3), and methanesulfonate esters (I-4), according to
the functional groups which they possess.

$$R-N\begin{cases} CH_2CH_2Cl \\ CH_2CH_2Cl \end{cases} \qquad R-N\begin{matrix} CH_2 \\ | \\ CH_2 \end{matrix} \qquad R-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-CH_3$$
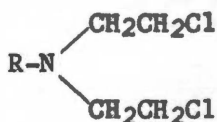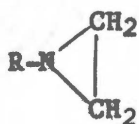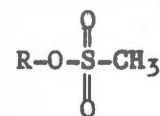
I-2   I-3   I-4

It is paradoxical that these medicinals should have their origin
in a war gas. Shortly after World War I Pappenheimer[4] and Pappenheimer
and Vance[5] published observations on the injection of bis-(2-chloroethyl)-
sulfide ("mustard gas") into rabbits. It was found that the injections
markedly diminished the number of leucocytes (e.g., white blood cor-
puscles). Hektoen and Corper[6] reported in 1920 that "mustard gas" pro-
foundly modified the leucocyte count of the blood in humans, dogs and
rabbits. Adair and Bragg[7] found that the surface application of bis-
(2-chloroethyl)sulfide in absolute alcohol controlled tar cancers in
mice. Berenblum[8] brought about the inhibition of the carcinogenic action
of tars by addition of "mustard gas" to the tar.

The explosion of a Liberty ship loaded with 100 tons of mustard
gas in the harbor of Bari, Italy during World War II played a signifi-
cant part in the treatment of cancer with chemical compounds. Since
leukemia, cancer of the blood system, is characterized by the excessive
formation of white corpuscles, the observation of profound depression
of the leucocyte count in the men rescued from the harbor prompted the
testing of bis-(2-chloroethyl)sulfide in leukemic patients. The effec-
tiveness of the compound was offset by its high toxicity and vesicant
action. Compounds structurally related to "mustard gas" with nitrogen

in place of sulfur ("nitrogen mustards") were then synthesized and tested.

The compound known specifically as nitrogen mustard, N-methyl-bis-(2-chloroethyl)amine hydrochloride, was prepared by Prelog and Stepan[9] in 1935. Satisfactory results in the treatment of Hodgkin's disease with this compound were reported by Goodman and Lewis[10] in 1946. It is now a standard drug for the treatment of leukemia and has been used to treat lymphosarcomas and carcinomas of the lung and ovary; however, the lethal dose of the drug is quite close to the effective dose.

Several hundred nitrogen mustards[11] have been synthesized and tested with the hope that a proper combination of the nitrogen mustard group with a second component would yield a drug having a high therapeutic index (e.g., ratio of lethal dose to effective dose). Nitrogen mustards based structurally on physiologically important compounds have shown promise in this respect. Good results have been claimed by the Russians for the phenylalanine mustard, 3-{p-[bis-(2-chloroethyl)amino]-phenyl}alanine (I-5), which was synthesized independently by Bergel and Stock[12] in England and by Larinov[13] in Russia. Chronic lymphatic leukemia and lymphocytic lymphoma have been treated successfully with chlorambucil, 4-{p-[bis-(2-chloroethyl)amino]phenyl}butyric acid (I-6), synthesized by Everett, Roberts, and Ross[14] at the Chester Beatty Research Institute.
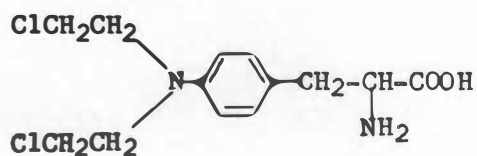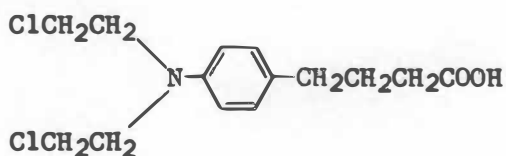
I-5   I-6

The physiological action of phenothiazine and its derivatives has been of considerable importance to the field of medicinal chemistry. Of particular interest has been the central nervous system effects of N-(dialkylaminoalkyl)phenothiazines.

Recently it has been demonstrated that two of the phenothiazine tranquilizers possess anticancer activity. Peters, Krais, and Gartner[15] reported that the treatment of mice bearing ascitic tumors with a combination of radium therapy and chloropromazine (I-7) brought about a

$$CH_3-N-CH_3$$
$$CH_2$$
$$CH_2$$
$$CH_2$$
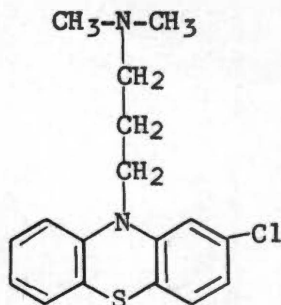
I-7

stronger effect in reducing the rate of cell-reproduction and inducing destruction of the tumor cells than treatment with either agent given alone. Fujita, Iwase, Ito, and Matsuyama[16] observed an inhibiting effect of chloropromazine on the experimental production of carcinoma of the liver in rats fed 4-dimethylaminoazobenzene. In experiments performed by Andrejew and Rosenburg[17] injections of chloropromazine significantly retarded development of tumor of the liver in rats but did not prevent eventual death. LeBlanc[18] reported a drop in the number of leucocytes in the blood of rats after injection of chloropromazine.

Compazine (I-8) has been shown by O'Malley, Wilheim, and Fluss[19]

$$N-CH_3$$
$$CH_2$$
$$CH_2$$
$$CH_2$$
$$N \qquad Cl$$
$$S$$
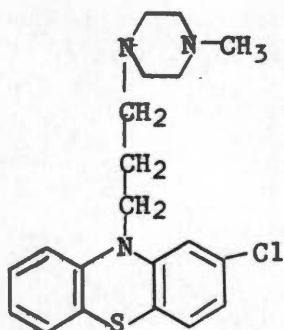
I-8

to increase the "built-in" ability of blood serum to destroy animal cancer cells. In one study tumor cells treated for 1 hour with Compazine and then transplanted into test mice did not produce new tumors in the animals. Tumor regression as well as the absence of cancer cells at autopsy was observed in another test with Compazine by the same workers.

In 1942 Badger, Elson, Haddow, Hewett, and Robinson[20] reported the inhibition of tumor growth by known carcinogens and closely related compounds. In the experiments the Walker carcinoma 256 was transplanted into two groups of 12 to 15 albino rats. Each animal in the trial group received an injection of a solution or suspension of the test compound. The control group was injected with the solvent. Two of the compounds which produced a significant inhibition of tumor growth were 7H-dibenzo-[c,h]phenothiazine and 14H-dibenzo[a,h]phenothizine, related structurally to the dibenzanthracenes. The 10-methyl derivative of 7H-benzo[c]-phenothiazine and 12-methylbenzo[a]phenothiazine also exhibited activity.

The evidence cited above for the physiological activity of the nitrogen mustards and the phenothiazine derivatives indicates that a

combination of these two types of molecules would be of interest. It
has been the main purpose of this research to synthesize a number of
N-(2-chloroethyl) and N-{2-[bis-(2-chloroethyl)amino]ethyl} derivatives
of phenothiazine (I-9), 7H-benzo[c]phenothiazine (I-10), 7H-dibenzo-
[c,h]phenothiazine (I-11) and 14H-dibenzo[a,h]phenothiazine (I-12) for
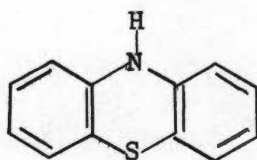
I-9
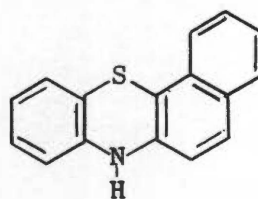
I-10
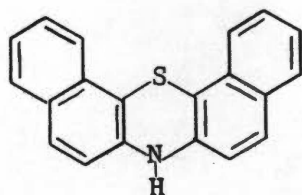
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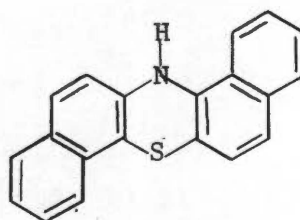
I-12

evaluation as potential cancer chemotherapeutic agents. The synthesis
of 14H-dibenzo[a,c]phenothiazine (I-13) and certain of its derivatives
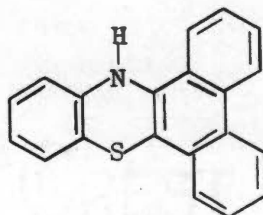
I-13

was also undertaken since there has previously been essentially no in-
formation in the literature on this heterocycle. The metalation of

the three dibenzophenothiazines mentioned above was studied to determine

the position of the entering metal atom. This reaction allows the in-

troduction of substituents into polynuclear, heterocyclic systems in

a highly selective manner.

CHAPTER II

HISTORICAL

A. Nomenclature

The compound known as phenothiazine was originally called
"thiodiphenylamine" by Bernthsen,[21] the father of phenothiazine chemistry.
Two other names, phenthiazine and 2,3:5,6-dibenzo-1,4-thiazine, have
appeared in the literature. The benzo- and dibenzophenothiazines were
also named originally as sulfur derivatives of diarylamines. The Ring
Index[22] has relieved some of the confusion in the nomenclature of these
compounds by listing not only the presently accepted name but also the
obscure designations found in the literature.

The numbering of the phenothiazine nucleus (II-1) used in this
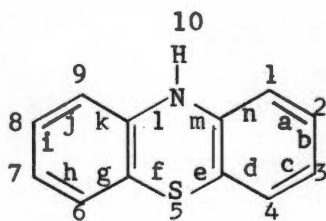
II-1

dissertation is that employed by Chemical Abstracts and the Ring Index.[22]
Another numbering (II-2) similar to that of anthracene is used in
Beilstein.[23]

9
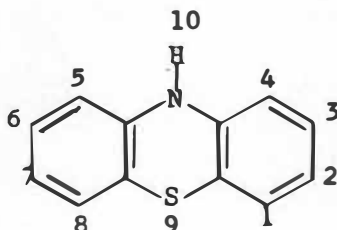
II-2

Originally the positions of fusion of benzene rings in benzo-
and dibenzophenothiazines were indicated by the numbers representing
adjacent positions on the phenothiazine nucleus. Besides being cumber-
some, this method necessitated a renumbering of the entire polycyclic
system to designate the positions of substitutents. This difficulty
is circumvented by lettering the peripheral sides of the phenothiazine
nucleus beginning with "a" for the side "1, 2," "b" for "2, 3," etc.
The name 3,4:6,7-dibenzophenothiazine then becomes 7H-dibenzo[c,h]-
phenothiazine.

Another problem in nomenclature involves atoms called "indicated
hydrogen." All of the known phenothiazines except two have derivatives[24]
(II-3) in which hydrogen is not on the nitrogen atom of the parent
compound but appears to be elsewhere in the molecule (II-4). Two or
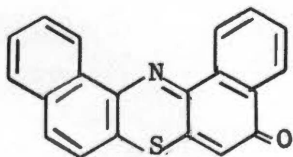
II-3
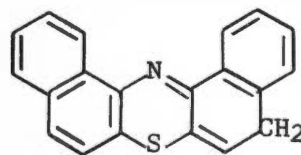
II-4

more isomers differing only in the position of a hydrogen atom must be
distinguished in some manner. The practice followed by <u>Chemical Abstracts</u>

and the Ring Index is the use of a prefix indicating the position of the hydrogen atom. The only exception to the rule among the compounds of interest in this thesis is phenothiazine itself in which case the hydrogen is indicated only when in a position other than "10."

B. Chemistry of Phenothiazine

1. Preparation

The earliest recorded synthesis of a phenothiazine compound can be traced back to 1876 when Lauth[25] heated p-phenylenediamine and sulfur. The action of ferric chloride on the hydrochloric acid solution of the reaction product produced a purple dye, Lauth's violet (II-5). In a similar manner Caro[26] synthesized methylene blue (II-6) from p-aminodimethylaniline in the same year.

II-5   II-6
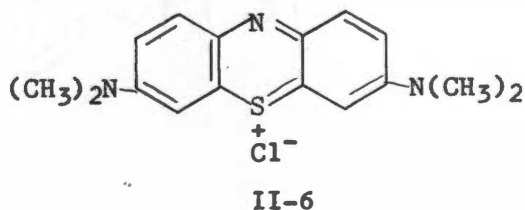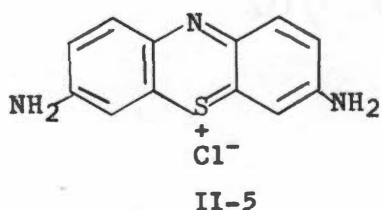
The structures of the two dyes were unknown until the work of Bernthsen showed that they were derivatives of phenothiazine. In 1883 Bernthsen[21] heated diphenylamine and sulfur at 250-300° until the evolution of hydrogen sulfide had ceased. He obtained a product which he called thiodiphenylamine. Nitration of the compound with fuming nitric acid produced two dinitrophenothiazine sulfoxide isomers. One of the isomers was reduced to 3,7-diaminophenothiazine, the oxidation of which yielded Lauth's violet.[27]

The reaction of diphenylamine and sulfur was greatly improved by Ackermann's introduction of a number of catalysts in a series of German patents. He accomplished the thionation of diphenylamine and its derivatives in 93 per cent yield and in a short time by the addition of aluminum chloride[28] to the reactants. The catalyst used most commonly today, iodine,[29] was mentioned in a second patent which also included aluminum bromide, aluminum iodide, ferric chloride, antimony trichloride, cuprous iodide and sulfur diiodide.

There have been a number of modifications employed in the synthesis of phenothiazine. Bernthsen[30] in 1885 obtained the compound from a mixture of diphenylamine, sulfur dichloride and sulfur. Holzman[31] produced phenothiazine in 41-45 per cent yield by the reaction of diphenylamine and sulfur dichloride in benzene. The reaction by Hofmann[32] of a mixture of aniline, aniline hydrochloride and sulfur at 195° yielded phenothiazine in small amounts. Geiger and Beck[33] in 1947 obtained a product of at least 95 per cent purity by sweeping out the hydrogen sulfide as it was formed in the thionation. Nitrogen, carbon dioxide, air and superheated steam were employed in this process.

The reactions and derivatives of phenothiazine are discussed extensively in a review by Massie[34] and in the doctoral dissertation (University of Virginia) of Schmalz.[35]

2. Nitrogen Mustard Derivatives

The simplest nitrogen mustard derivative of phenothiazine, 10-(2-chloroethyl)phenothiazine, was prepared by Spatz and Kolbezen[36] and subsequently by Gilman and Shirley[36] who used the compound as an inter-

mediate in the synthesis of dialkylaminoalkyl derivatives of pheno-
thiazine. The preparation of the compound involved the reaction of
Phenothiazine with n-butyllithium to produce 10-lithiophenothiazine
followed by the addition of 2-chloroethyl p-toluenesulfonate.

A recent abstract of a French patent[37] reports the preparation
of a series of eleven phenothiazine nitrogen mustards. Ten of these
compounds are of the type 10-[N-(2-chloroethyl)aminoalkyl]phenothiazine
(II-7) in which only one 2-chloroethyl group is attached to a nitrogen

$$R-N-CH_2CH_2Cl$$
$$(CH_2)_x$$
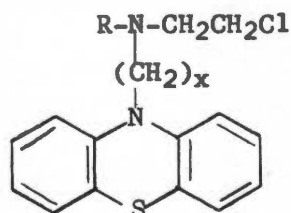
II-7

atom. The remaining compound is 2-chloro-10-{3-[bis-(2-chloroethyl)-
amino] propyl}phenothiazine, the nitrogen mustard analog of chloro-
promazine (I-7). A typical synthesis involved the preparation of 10-
[2-(2-hydroxyethylethylamino)ethyl]phenothiazine in 85 per cent yield
by heating 70 g. of 10-(2-chloroethyl)phenothiazine with 180 g. of
ethylaminoethanol and 7.5 g. of powdered copper for 20 hours at 100°.
Conversion of the hydroxy compound to the mustard hydrochloride was
accomplished with thionyl chloride in benzene in a yield of 54 per
cent. No published reports of tests of these materials for anticancer
activity have been found.

C. Chemistry of the Benzophenothiazines

1. 12H-Benzo[a]phenothiazine

In 1890 Kym[38] reported the preparation of 12H-benzo[a]pheno-
thiazine by the thionation of N-phenyl-1-naphthylamine at 210-240° for
6 hours. Knoevenagel[39] and Kehrman and Dardel[40] improved the synthesis
by the use of iodine as the catalyst for the reaction. A German
patent[41] described a procedure for the preparation of 12H-benzo[a]pheno-
thiazine from a mixture of aniline, 1-naphthylamine, sulfur and iodine.

The chemistry of 12H-benzo[a]phenothiazine has been investigated
in this laboratory by Talukdar and Shirley[42] and by Waters.[43] Talukdar
and Shirley[42] obtained a yield of 80 per cent of 12H-benzo[a]pheno-
thiazine melting at 135-137° by heating a mixture of N-phenyl-1-
naphthylamine, sulfur and a small amount of iodine at 180-185° for 25
minutes. Nuclearly substituted 12H-benzo[a]phenothiazines were prepared
in a similar manner by the same workers from substituted N-phenyl-1-
naphthylamines. Several 12-(dialkylaminoalkyl)benzo[a]phenothiazine
analogs of the phenothiazine tranquilizers were prepared by Talukdar
and Shirley[42] for pharmacological evaluation.

Using procedures essentially the same as those described in a
recent publication by Shirley, Sen, and Gilmer,[44] Waters[43] prepared 12-
(2-chloroethyl)- and 12-{2-[bis-(2-chloroethyl)amino]ethyl}benzo[a]-
phenothiazine for testing by the Cancer Chemotherapy National Service
Center. A literature survey of the derivatives and reactions of 12H-
benzo[a]phenothiazine can be found in the thesis of Waters.[43]

2. 7H-Benzo[c]phenothiazine

The synthesis of 7H-benzo[c]phenothiazine was described in 1890 by Kym[38] who obtained the compound from the reaction of N-phenyl-2-naphthylamine and sulfur. The reaction was facilitated by the use of iodine as catalyst, employed first by Knoevenagel[39] and later by Kehrman and Dardel.[40] The latter investigators carried out the reaction at reduced pressure (12-15 mm.) at a temperature of 200° for 2 hours and obtained a yield of 80 per cent.

Tatum[45] and Schmalz[35] reported difficulty in repetition of previous procedures for the preparation of 7H-benzo[c]phenothiazine. Tatum[45] in this laboratory studied various thionation methods and found that reaction of an intimate mixture of N-phenyl-2-naphthylamine, sulfur, and iodine at 175-180° for 20 minutes gave the best results. He reported yields of 46-61 per cent and a melting point of 180-181° for material recrystallized from benzene.

Shirley and Tatum[45,46] prepared a series of 7-(dialkylaminoalkyl)-benzo[c]phenothiazines similar to the active phenothiazine tranquilizers. The synthesis of these compounds involved the reaction of 7H-benzo[c]-phenothiazine or one of its derivatives with sodamide followed by the addition of a dialkylaminoalkyl chloride. The products were purified by distillation in vacuo.

Little is known about substitution reactions on the nucleus of 7H-benzo[c]phenothiazine. Schmalz[35] carried out the nitration and formylation of the nucleus, but did not determine the positions taken by the entering groups.

Recently Shirley and Gopalreddy[47] investigated the Friedel-Crafts acylation of 7-acetylbenzo[c]phenothiazine. Earlier work by Baltzly, Harfenist, and Webb[48] had shown that reaction of 10-acetyl-phenothiazine with acetyl chloride and aluminum chloride at the boiling point of carbon disulfide yielded 2,10-diacetylphenothiazine. With an excess of acetyl chloride Michels and Amstutz[49] obtained 2,8,10-tri-acetylphenothiazine. Burger and Clements[50] in 1954 reported the acyla-tion of 7-acetylbenzo[c]phenothiazine but did not establish the structure of the product. Shirley and Gopalreddy[47] repeated this work and obtained 7,X-diacetylbenzo[c]phenothiazine, m.p. 203-204°. The compound was oxidized by a modified iodoform reaction to X-carboxy-7H-benzo[c]pheno-thiazine, m.p. 156-157° with decomposition. Wolff-Kishner reduction of X-acetyl-7H-benzo[c]phenothiazine gave X-ethyl-7H-benzo[c]phenothiazine, m.p. 208-209° with decomposition. The 8, 10, and 9 (11)*-ethyl deriva-tives of 7H-benzo[c]phenothiazine were prepared but the X-ethyl compound differed from each of these. The X-carboxy derivative was shown to be different from 6-carboxy-7H-benzo[c]phenothiazine prepared previously by Tatum.[45]

At this point the information is of a negative nature, excluding the 6, 8, 9 (11) and 10 positions as sites of acylation. It seems likely that the reaction occurred at the 5-position of 7H-benzo[c]phenothiazine (II-8) since this corresponds to the 2-position of phenothiazine.

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*Thionation of N-(m-ethylphenyl)-2-naphthylamine could give either the 9- or 11-isomer.
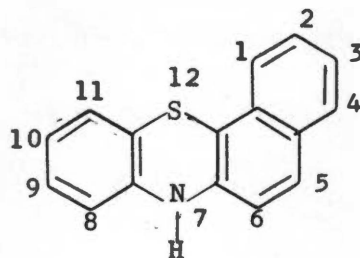
II-8

A complete literature survey of the chemistry of 7H-benzo[c]-
phenothiazine from its original synthesis to 1958 is contained in the
doctoral dissertation of Tatum.[45]

3. 12H-Benzo[b]phenothiazine

The information in the literature on the third isomer, 12H-
benzo[b]phenothiazine (II-9) is rather limited. Fries and Kirkow[51]
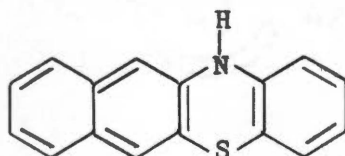
II-9

prepared the compound in 1922 as one of a series of studies by Fries
on the chemistry of 2,3-dichloro-1,4-naphthoquinone. A derivative of
the latter compound, 2-anilino-3-mercapto-1,4-naphthoquinone, was
oxidized with air in boiling alcohol. The product of the reaction was
12H-benzo[b]phenothiazine-6,11-dione (II-10) which, when reduced by
warming for 1 hour with a large excess of stannous chloride in glacial
acetic acid, yielded 12H-benzo[b]phenothiazine, m.p. 277°. Less vigorous
reduction with stannous chloride yielded 6,11-dihydroxy-12H-benzo[b]-
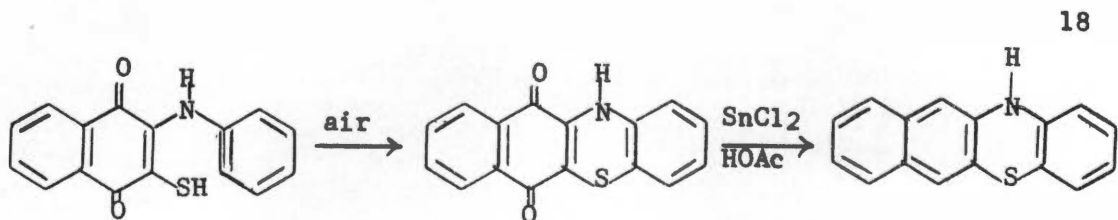
II-10

phenothiazine. Fries and Kohler[52] have reported the synthesis of additional derivatives of 12H-benzo[b]phenothiazine-6,11-dione.

D. Chemistry of the Dibenzophenothiazines

1. 7H-Dibenzo[c,h]phenothiazine

Ris[53] in 1886 reported the original synthesis of 7H-dibenzo[c,h]-phenothiazine. The compound, which he called thio-β-dinaphthylamine, was produced by the reaction of a mixture of di-2-naphthylamine and sulfur at 250° for 10 hours. The yellow product melted at 236° after recrystallization from benzene. Ris[53] heated 7H-dibenzo[c,h]phenothiazine with copper powder in a carbon dioxide atmosphere and 7H-dibenzo[c,g]carbazole (II-11), m.p. 170°, distilled from the mixture.
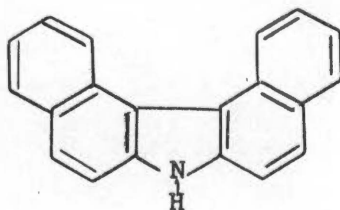
II-11

The melting point observed by Ris for 7H-dibenzo[c,g]carbazole was ca. 10 degrees higher than that reported earlier by Walder.[54] However, Ris[53] prepared a picrate derivative of the compound, the melting point of which agreed with the value of the picrate prepared by Walder.[54]

Ris[53] also reported the conversion of 7H-dibenzo[c,h]phenothiazine to 7H-dibenzo[c,h]phenoxazine (II-12) by means of a sealed tube reaction
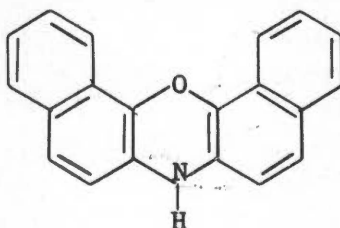
at 250-280° for 2-3 hours using a copper-copper oxide catalyst. A structure proof for the dibenzophenoxazine was not given.

In 1888 Kym[55] synthesized 7H-dibenzo[c,h]phenothiazine by the treatment of di-2-naphthylamine with sulfur dichloride in benzene. The first mention of the use of iodine as a catalyst for the preparation of 7H-dibenzo[c,h]phenothiazine was contained in a German patent[41] involving the reaction of 2-naphthylamine, sulfur and iodine. Shortly after the appearance of the patent Knoevenagel[39] gave a detailed description of the reaction. He heated a mixture of 2-naphthylamine, sulfur and a small amount of iodine at a bath temperature of 200° for 2.5 hours and obtained an 80 per cent yield of material melting at 228°. Repeated recrystallization from benzene raised the melting point to 236°. Knoevenagel[39] also reported yields of 80-90 per cent for the preparation of 7H-dibenzo[c,h]phenothiazine from sulfur and di-2-naphthylamine with or without iodine. Later Kehrman and Christopoulos[56] also obtained a yield of 80 per cent by heating di-2-naphthylamine and sulfur for 10 minutes at 200-210°.

In 1937 Fang, Liu and Sah[57] reported the reaction of di-2-naphthylamine, sulfur and a few iodine crystals. The mixture was heated in a

bath at 190° until the reactants solidified (5 minutes). Yellow needles, m.p. 222-223°, were obtained from toluene in almost quantitative yield. The structure for the product of the reaction listed in the index of Chemical Abstracts and given in the abstract in Chemisches Zentralblatt corresponds to 13H-dibenzo[b,i] phenothiazine (II-13). The designations "β-naphththiazine" and "thiodi-β-naphthyl-
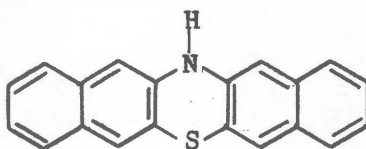
II-13

amine" are used in both abstracts. The Ring Index[22] lists "β-napthiazine" as another name for 13H-dibenzo[b,i] phenothiazine and "thio-β-dinaphthylamine" as a name for 7H-dibenzo[c,h] phenothiazine. The contradictions in nomenclature in both abstracts are apparently due to the naming of the compound by Fang, Liu and Sah.[57] The product of the reaction must have been 7H-dibenzo[c,h] phenothiazine, since the reactants and conditions employed were essentially the same as those used earlier by Knoevenagel.[39] The Chinese workers[57] formed a picrate derivative, m.p. 250-251°, which checks well with the picrate of 7H-dibenzo[c,h] phenothiazine obtained by Ris.[53]

A literature search for derivatives of 7H-dibenzo[c,h] phenothiazine revealed only one nuclear substitution at a position other than 7 (N) or 14 (S). Scholl and Seer[58] in 1911 reported the reaction of 7H-dibenzo[c,h] phenothiazine, phthalic anhydride, and aluminum chloride at 145°. The crude material obtained from the reaction was

cyclized by heating at 140° with concentrated sulfuric acid. No physical constants or structure were given for the black product which the investigators called "thiodinaphthanthraquinonylamine." The same workers performed a similar transformation on phenothiazine and obtained a product which they called thiodianthraquinonylamine (II-14). Similar
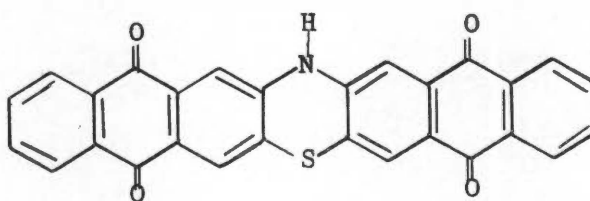
II-14

structures can be drawn for the reaction of 7H-dibenzo[c,h]phenothiazine (II-15, II-16) but they do not seem to correspond to the nomenclature used by Scholl and Seer.[58]
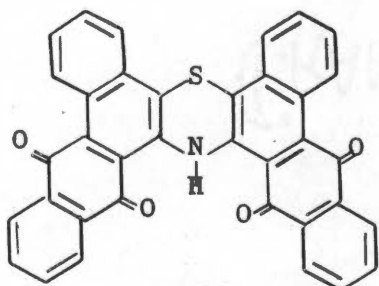
II-15
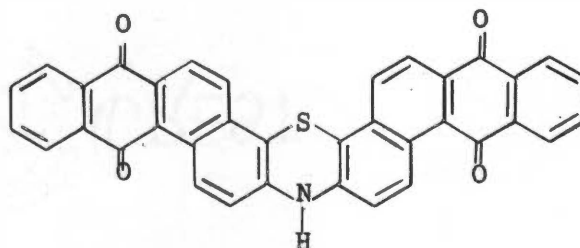
II-16

A number of N-alkyl and N-acyl derivatives of 7H-dibenzo[c,h]-phenothiazine have been prepared. Few instances of the formation of N-substituted phenothiazines by thionation of tertiary amines are recorded. However, Kym[38] employed this unusual method for the synthesis of 7-methyl- and 7-ethyldibenzo[c,h]phenothiazine. Reaction of N-methyl-di-2-naphthylamine and sulfur at 230-240° for 5 hours yielded the 7-methyl derivative, m.p. 284-285°. A satisfactory yield also was obtained by

the reaction of the same amine with sulfur dichloride. His third preparation of the methyl compound involved a sealed tube reaction of 7H-dibenzo[c,h]phenothiazine and methyl iodide in methyl alcohol at 150° for 5 hours. Kym[38] prepared 7-ethyldibenzo[c,h]phenothiazine, m.p. 212-213°, from N-ethyl-di-2-naphthylamine and sulfur dichloride and from 7H-dibenzo[c,h]phenothiazine and ethyl iodide in a sealed tube reaction similar to the one described above.

A series of interesting carbonyl derivatives was prepared by Paschkowetzky.[59] The reaction of 7H-dibenzo[c,h]phenothiazine with phosgene in toluene at 160-170° yielded the N-chlorocarbonyl derivative, which when allowed to react with sodium phenoxide, ammonia, aniline or 7H-dibenzo[c,h]phenothiazine formed the products II-17, II-18, II-19, and II-20. In the figure below Z-NH represents 7H-dibenzo[c,h]phenothiazine.
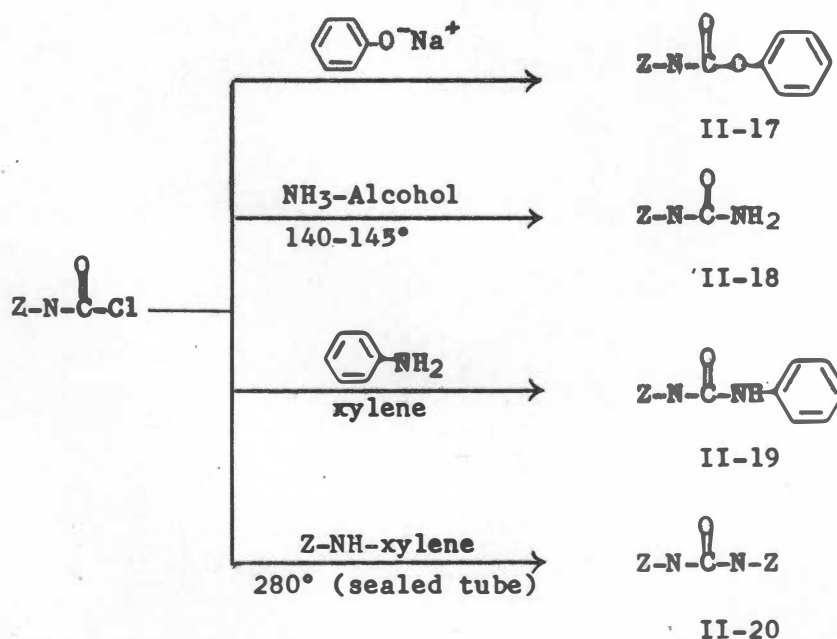
A few reactions involving the sulfur atom of 7H-dibenzo[c,h]-phenothiazine are described in the literature. Kehrman and Dardel[40] found that reaction of the dibenzo compound with dimethyl sulfate followed by treatment with perchloric acid gave 7,14-dihydro-14-methyl-dibenzo[c,h]phenazathionium perchlorate (II-21) in 55 per cent yield.
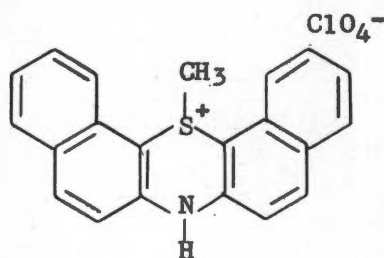
II-21

Sodium hydroxide converted an aqueous solution of the methyl sulfate salt to 14H-14-methyldibenzo[c,h]phenothiazine (II-22), m.p. 160-165°.
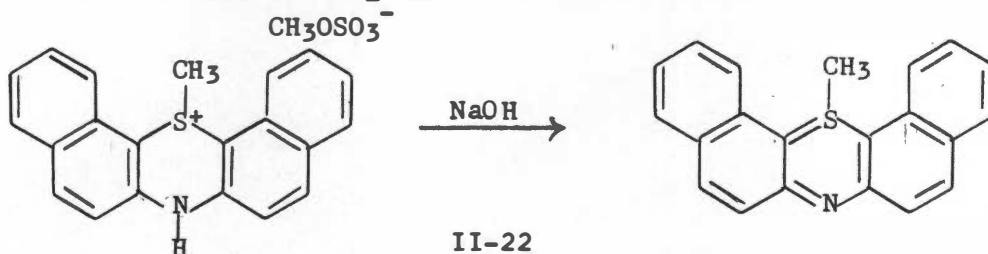
II-22

The 7,14-dihydro-7,14-dimethyldibenzo[c,h]phenazathionium salt was prepared by the same workers in a similar manner from 7-methyldibenzo[c,h]-phenothiazine and dimethyl sulfate.

Kehrman and Christopoulos[56] reported the formation of 7H-dibenzo-[c,h]phenothiazine-9-oxide by oxidation of the parent compound with sodium nitrite. The colorless needles decomposed at 212° without melting.

2. 14H-Dibenzo[a,j]phenothiazine

The original preparation of 14H-dibenzo[a,j]phenothiazine was reported by Kehrman, Misslin and Gressly[60] in 1902. A mixture of

di-1-naphthylamine and sulfur was heated for 6 hours at a temperature of 230-240° and orange-yellow plates, m.p. 176-177°, were obtained. Knoevenagel's[39] introduction in 1914 of iodine as a catalyst for the reaction resulted in yields of 50-60 per cent of pure material, m.p. 176°, from the reaction of amine, sulfur and iodine for only 9 minutes at 163°. Later Kehrman and Dardel[40] reported yields of 60-70 per cent using iodine as catalyst and a reaction time of 2 hours. Kehrman, Gressly, Chiffere and Ramm[61] found that 14H-dibenzo[a,j]phenothiazine melted at 164-166° in a sealed tube under an atmosphere of carbon dioxide.

Recently Waters[43] attempted the synthesis of the compound. He experienced difficulty with purification of the easily oxidized reaction product. Previous descriptions of 14H-dibenzo[a,j]phenothiazine[40,61] had indicated that oxidation occurred readily, especially in solution.

Only a few derivatives of 14H-dibenzo[a,j]phenothiazine are known. Kehrman and Misslin[24] formed 5H-dibenzo[a,j]phenothiazin-5-one (II-23) by oxidation of the parent compound with ferric chloride in the presence of aniline hydrochloride followed by hydrolysis of the anilino compound with water.

II-23
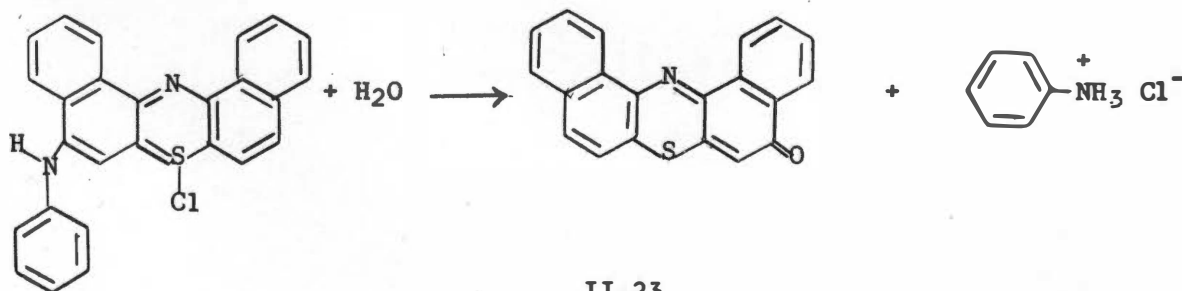
Kehrman and Dardel[40] prepared 7,14-dihydro-7-methyldibenzo[a,j]-phenazathionium perchlorate by the reaction of 14H-dibenzo[a,j]phenothiazine with dimethyl sulfate followed by treatment with perchloric acid. Sodium hydroxide converted the colorless salt to 7H-7-methyl-dibenzo[a,j]phenothiazine (II-24), which was obtained as golden yellow needles, m.p. 141°.
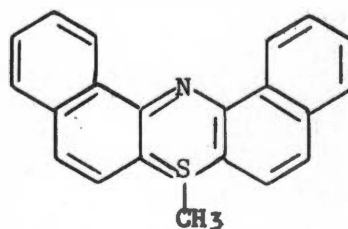
II-24

The only N-substituted derivative found in the literature is the 14-acetyl derivative prepared by Kehrman, Gressly, Chiffere and Ramm[61] from a mixture of 14H-dibenzo[a,j]phenothiazine, acetic anhydride and zinc chloride. The same workers prepared the nitrate, perchlorate and chloroferrate salts of 14H-dibenzo[a,j]phenothiazine but reported no physical constants.

3. 14H-Dibenzo[a,h]phenothiazine

The first mention of 14H-dibenzo[a,h]phenothiazine is in a 1923 paper by Kehrman, Gressly, Chiffere and Ramm.[61] However, the authors indicated that the preparation had been described 20 years earlier in a dissertation by Gressly. A mixture of 1,2'-dinaphthylamine and sulfur was heated for 1.5-2 hours at 240°. Orange-yellow prisms, m.p. 185-186°, were obtained from benzene. The same workers prepared the nitrate, picrate, and perchlorate salts but did not report their melting

points. They also formed 5H-dibenzo[a,h]phenothiazin-5-one, m.p. 256-257°, by the oxidation of 14H-dibenzo[a,h]phenothiazine with ferric chloride in a mixture of water and acetic acid.

Several N-substituted derivatives of the compound were prepared by Waters.[43] He obtained the 14-methyl, 14-(2-chloroethyl), and 14-[2-(diethylamino)ethyl] derivatives by the reaction of 14H-dibenzo-[a,h]phenothiazine with n-butyllithium, followed by the addition of the appropriate sulfonate ester or alkyl chloride. The 14-acetyl derivative, m.p. 191-193°, was obtained by the action of acetyl chloride and zinc chloride on 14H-dibenzo[a,h]phenothiazine. The acetyl sulfone derivative, m.p. 240-241°, was prepared by oxidation of 14-acetyldibenzo-[a,h]phenothiazine with hydrogen peroxide in glacial acetic acid. The acetyl group was removed in boiling alcoholic sodium hydroxide to yield 14H-dibenzo[a,h]phenothiazine-7-dioxide which melted above 405°.

4. 13H-Dibenzo[b,i]-, 5H-Dibenzo[a,i]- and 7H-Dibenzo[b,h]phenothiazine

Essentially all of the information in the literature concerning these three isomers is contained in the work of Fries on the reactions of 2,3-dichloro-1,4-naphthoquinone. In 1923 Fries and Ochwat[62] reported the reaction (II-25) of 2-anilino-3-mercapto-1,4-naphthoquinone with
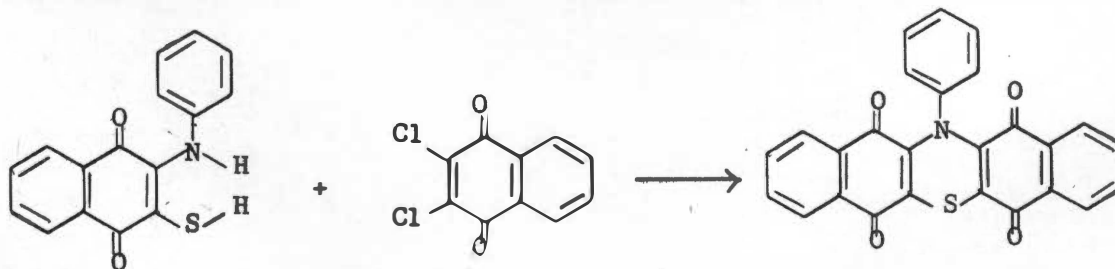
II-25

2,3-dichloro-1,4-naphthoquinone. The product, 13-phenyldibenzo[b,i]-
phenothiazine-5,7,12,14-tetrone, in boiling nitrobenzene or in acetic
acid containing a small amount of nitric acid was converted to the cor-
responding carbazole (II-26).
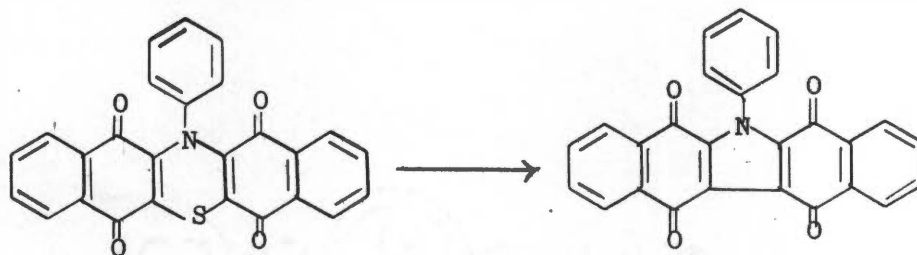
II-26

The reaction of 2-amino-3-mercapto-1,4-naphthoquinone with 2-
amino-3-chloro-1,4-naphthoquinone by Fries and Ochwat[62] resulted in the
formation of a compound, the elemental analysis of which corresponded
to $C_{20}H_{10}N_2O_3S$. A structure (II-27) corresponding to 5H-6-amino-
dibenzo[a,i]phenothiazine-5,8,13-trione was proposed for the brownish
yellow material which melted above 360°.
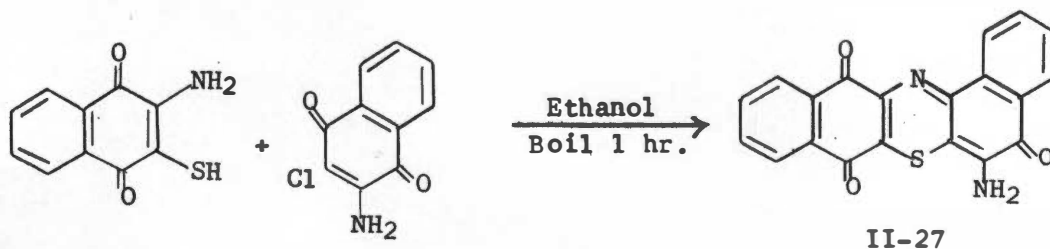
II-27

A synthesis similar to that of 12H-benzo[b]phenothiazine-6,11-
dione yielded the quinone of 7H-dibenzo[b,h]phenothiazine.[52] The inter-
mediate, 2-(2'-naphthylamino)-3-mercapto-1,4-naphthoquinone, was pre-
pared by reaction of 2,3-dichloro-1,4-naphthoquinone with 2-naphthyl-
amine followed by conversion with sodium sulfide to the mercapto de-
rivative. Air oxidation of the intermediate in boiling alcohol resulted

in the formation of 7H-dibenzo[b,h] phenothiazine-8,13-dione (II-28).

II-28
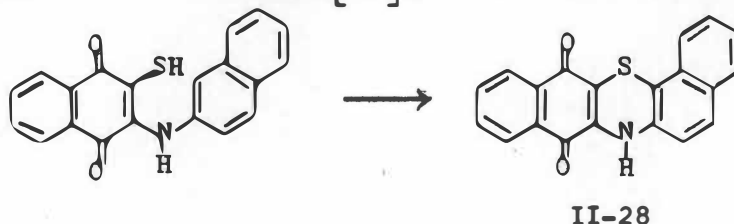
The product was obtained in the form of deep green-blue crystals, m.p. 300°.

Another reference to 7H-dibenzo[b,h]- and 13H-dibenzo[b,i]-phenothiazine is taken from the work of Kym.[55] His treatment of 2-dinaphthylamine with sulfur dichloride in benzene produced 7H-dibenzo-[c,h]phenothiazine as the main product. However, he also isolated from the reaction mixture a small amount of a compound the elemental analysis of which showed it to be a new dibenzophenothiazine. The almost white, microscopic needles were sparingly soluble in benzene and melted at ca. 289°. A sample placed in a bath at 280° did not melt until the temperature had reached 307°. Kym did not propose a structure but the editors of Beilstein[63] logically indicated that the compound was either 7H-dibenzo[b,h] phenothiazine or 13H-dibenzo[b,i] phenothiazine.

5. 14H-Dibenzo[a,c]phenothiazine

Only one reference to 14H-dibenzo[a,c]phenothiazine (II-29) was found in the literature. Pacault[64] in 1946 reported the results of a

II-29
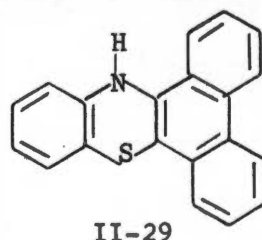
study of magnetic susceptibilities of a large number of compounds. Included in the list were phenothiazine and "dibenzo-1,2,3,4-phenothiazine." However, no record of the synthesis or physical constants of the compound could be uncovered. The latest edition of the Ring Index[22] lists 14H-dibenzo[a,c] phenothiazine, but cites the work of Pacault, described above, as the only reference.

E. Metalation of the Phenothiazines

The original investigations of the metalation of phenothiazine were conducted by Gilman, Shirley and Van Ess.[65,66] They found that the reaction of phenothiazine and n-butyllithium followed by the addition of solid carbon dioxide to the mixture gave a 52 per cent yield of 1-carboxyphenothiazine[65] (II-30).

1) n-C4H9Li
2) CO2
3) H3O+
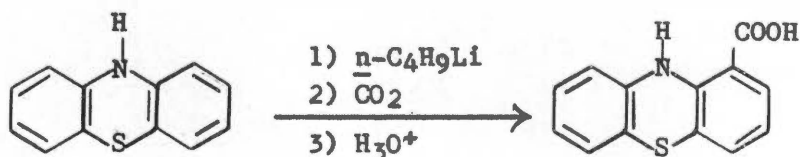
II-30

A different orientation was shown to prevail in N-substituted phenothiazines.[66] The same workers obtained poor yields of monocarboxylic acids (II-31, II-32) from the metalation and carbonation of
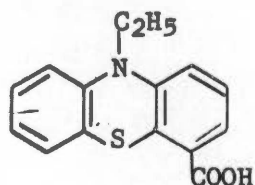
II-31
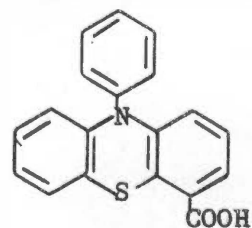
II-32

10-ethyl- and 10-phenylphenothiazine. Degradation of the carboxylic acid derivative of 10-ethylphenothiazine with hydroiodic acid yielded 3-carboxydiphenylamine (II-33) which would be produced from either the

II-33
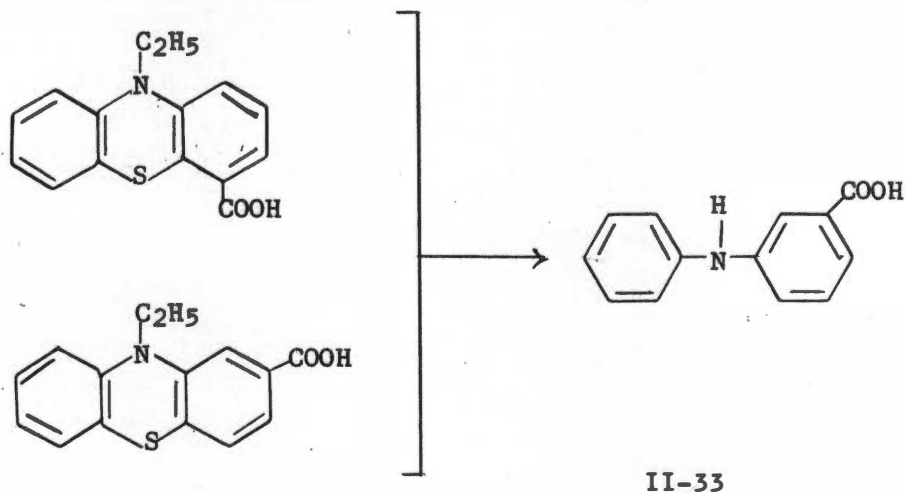
2- or the 4-carboxy isomer. In a similar manner the 10-phenyl acid was degraded to 3-carboxytriphenylamine. Since previous work[67,68] had shown the tendency for metalation to occur ortho to a hetero atom, the structure corresponding to 4-carboxy-10-ethylphenothiazine was assigned to the acid. Later work[48,49] on the Friedel-Crafts acylation of 10-acetylphenothiazine has shown that the proposed structure was correct.

The investigation of the metalation of 10-methylphenothiazine with n-butyllithium by Goan[69] also demonstrated orientation in the 4-position. 4-Carboxy-10-methylphenothiazine was obtained in a yield of 43 per cent.

The metalation of 10-ethylphenothiazine-5-oxide with n-butyllithium by Nelson[70] resulted in the formation of 4-carboxy-10-ethyl-phenothiazine.

Recent studies in this laboratory have shown that 7H-benzo[c]-phenothiazine is metalated in a manner similar to phenothiazine. Tatum[45] found that metalation of 7H-benzo[c]phenothiazine with n-butyllithium followed by carbonation yielded 6-carboxy-7H-benzo[c]phenothiazine (II-34), m.p. 300-301°. The naphthalene ring rather than the
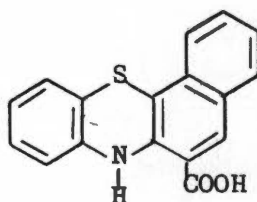
II-34

benzene ring was the site of reaction. The structure of the acid was proved by desulfurization with Raney nickel to N-phenyl-3-amino-2-naphthoic acid followed by cyclization with polyphosphoric acid to benz[b]acridone. A small amount of an orange solid melting at 264-266° with decomposition was also isolated from the metalation reaction. The elemental analysis of the latter compound indicated that it was a monocarboxylic acid derivative of 7H-benzo[c]phenothiazine. The infrared spectrum of the compound was quite similar to that of 6-carboxy-7H-benzo[c]phenothiazine.

In 1954 Schmalz[35] attempted the metalation of 7H-benzo[c]-phenothiazine with n-butyllithium. He obtained bright orange crystals, m.p. 275-276°, the elemental analysis of which corresponded to the formula $C_{17}H_{11}NO_2S$. The position of metalation was not determined and attempts to reproduce the formation of the acid with n-butyllithium and with phenylsodium were unsuccessful.

Shirley and Liu[71] observed an unusual orientation in the metala-
tion (II-35) of 12H-benzo[a]phenothiazine. The 1-position was found
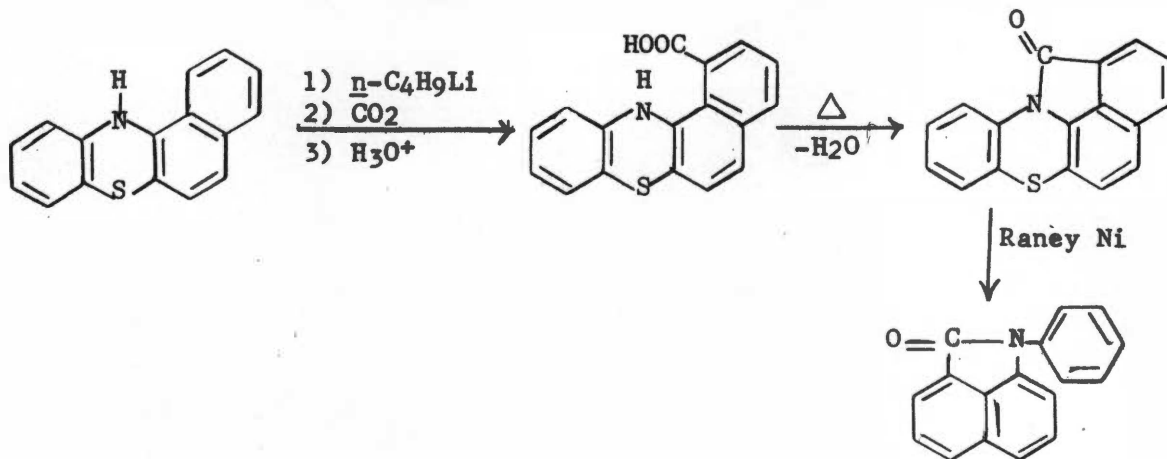
II-35

to be the reaction site. The acid was converted by heating at 165°
for several minutes to the lactam, 12-oxo-12H-isoindolo[2,1,7-m,n,a]-
phenothiazine. Desulfurization of the lactam with Raney nickel re-
sulted in the formation of N-phenylnaphthastyril which was shown to
be identical with a sample of N-phenylnaphthastyril from an independent
synthesis.

DISCUSSION

A. Introduction

The primary purpose of this research has been the synthesis of some nitrogen mustard type derivatives of the phenothiazines. The pursuit of this main objective necessitated a study of the synthesis of some of the phenothiazines, as well as the N-substituted intermediates leading to the nitrogen mustards. The reaction series given below using 7H-dibenzo[c,h]phenothiazine illustrates the path followed.
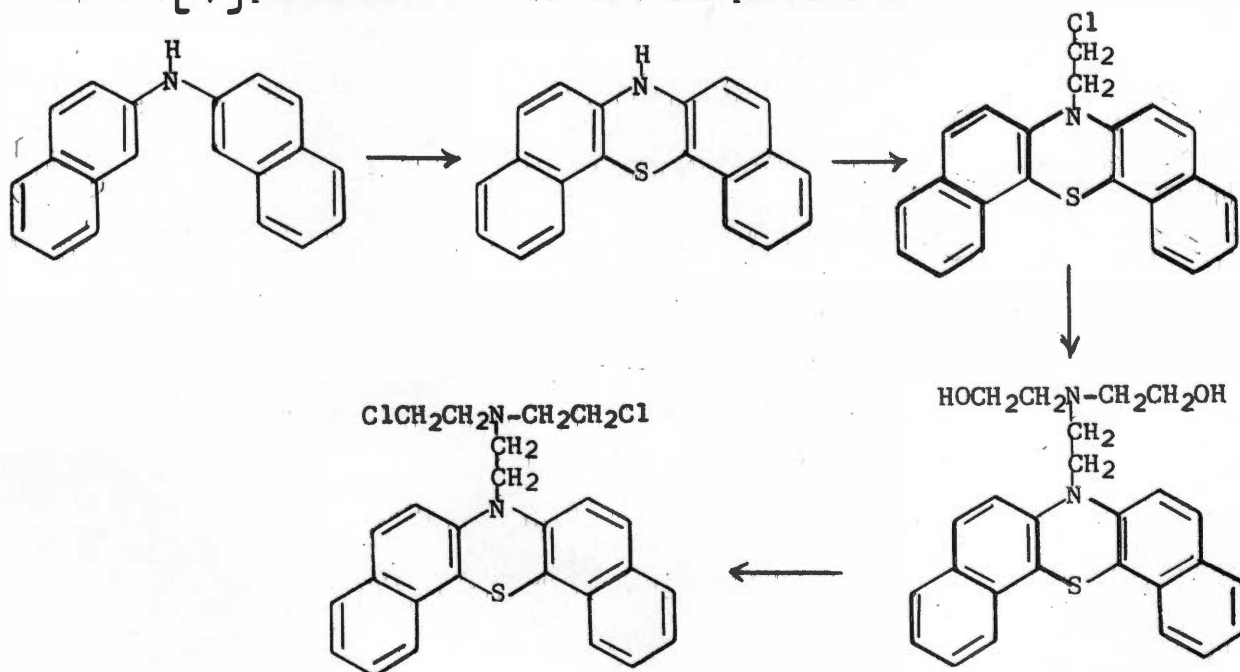
In the first step a secondary amine undergoes thionation to form the nitrogen and sulfur containing heterocyclic ring system. The condensation of diethanolamine with the N-(2-chloroethyl) derivative, formed in

the second step by the reaction of an N-lithiophenothiazine with 2-chloroethyl p-toluenesulfonate, yields the N-{2-[bis-(2-hydroxyethyl)-amino]ethyl}derivative. The final step involves the conversion of the hydroxy compound to the nitrogen mustard with phosphorus oxychloride.

B. Synthesis of Phenothiazines

1. Diaryl Amines

The diarylamines used in the synthesis of 7H-benzo[c]phenothiazine and 7H-dibenzo[c,h]phenothiazine were obtained commercially. N-Phenyl-2-naphthylamine was purchased from Eastman Organic Chemicals and di-2-naphthylamine from the Aldrich Chemical Company.

The intermediate in the formation of 14H-dibenzo[a,h]phenothiazine, 1,2'-dinaphthylamine, was kindly provided by W. D. Waters.[43] A small amount of 12H-benzo[a]phenothiazine was also supplied by Waters.

Little information was available in the literature on N-phenyl-9-phenanthrylamine needed for the preparation of 14H-dibenzo[a,c]-phenothiazine. The abstracts of a German patent[72] issued in 1937 indi-cated that Wolfram and Schnurr had prepared the amine, m.p. 138°, by the reaction of sodium anilide and 9-chlorophenanthrene.

An investigation of the synthesis and purification of N-phenyl-9-phenanthrylamine was necessary. A procedure similar to one used by Scardiglia and Roberts[73] for the preparation of diphenylamine from potas-sium anilide and bromobenzene gave good results when applied to potassium anilide and 9-bromophenanthrene. Potassium metal was added slowly to re-fluxing aniline and the resulting black solution was stirred under reflux

for several hours. 9-Bromophenanthrene then was added and the mixture was stirred under reflux for several hours longer. The yields for 4 such reactions were in the range 37-42 per cent. Variations in the time of reaction of potassium and aniline and of potassium anilide and 9-bromophenanthrene did not affect the yield.

2. Thionation of Diarylamines

The syntheses of 7H-benzo[c]phenothiazine,[45] 7H-dibenzo[c,h]-phenothiazine,[39] and 14H-dibenzo[a,h]phenothiazine[43] have been studied and reported previously. However, it was observed in this research, and also has been the experience of other investigators in this Laboratory,[42,43,45] that the duplication of the conditions and yields described for these reactions is quite difficult. The primary variables in such syntheses are the temperature at which the reaction is initiated and the rate of heating from the initial to the final temperature. The purity of the amine used is also an important factor. It has generally been necessary in the thionation reaction for the investigator to modify an existing procedure in order to obtain the best results from his own particular techniques.

Tatum[45] prepared 7H-benzo[c]phenothiazine in yields of 46-61 per cent by the reaction of N-phenyl-2-naphthylamine, sulfur and iodine at 175-180° for 20 minutes. It was found that yields similar to those obtained by Tatum could be produced by placing the mixture of reactants in a Wood's metal bath previously heated to a temperature of 175° and maintaining the temperature at 175-180° for 35 minutes.

The reaction of di-2-naphthylamine, sulfur and iodine for 10 minutes at 190° was reported by Knoevenagel[39] to yield 80-90 per cent of 7H-dibenzo[c,h] phenothiazine. However, the same yields were obtained in this research with a lower temperature and a shorter reaction time. The reactants melted about 140° and immediately resolidified forming a cake, the surface of which was covered with a thin layer of a black impurity. The bright yellow powder obtained when the cake was crushed was recrystallized once from benzene to yield yellow needles, m.p. 222-225°. A product from the reaction of 2-naphthylamine, sulfur and iodine required more purification than that produced from di-2-naphthylamine, since the much longer thionation time (3 hours) of 2-naphthylamine allowed the formation of impurities.

A melting point of 228-230° for 7H-dibenzo[c,h] phenothiazine was the highest obtained in this work. Ris,[53] Kym[55] and Knoevenagel[39] had reported a melting point of 236° for the compound. The black melt obtained at 228-230° obviously contained decomposition products which probably were formed by oxidation. Inability to duplicate the conditions under which the melting point of 236° was obtained probably accounted for the difference in the point of decomposition. Evidence in support of this statement is the fact that a sample of 7H-dibenzo[c,h] phenothiazine in an evacuated capillary melted at 245-246°, yielding a clear, amber liquid.

Waters' synthesis[43] of 14H-dibenzo[a,h] phenothiazine was utilized with essentially no alteration.

The thionation of N-phenyl-9-phenanthrylamine, which had not
previously been reported, produced small yields of 14H-dibenzo[a,c]-
phenothiazine (III-1). The effects of variations in the time and

III-1
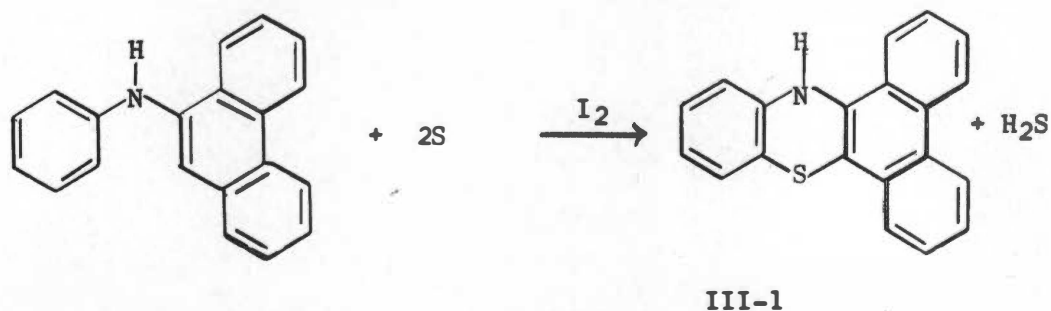
temperature of the reaction were studied, but the best yield obtained
was only 16 per cent. Attempts to isolate 14H-dibenzo[a,c]phenothiazine
by crystallization of the reaction mixture were unsuccessful. Chroma-
tographic separation of benzene-ligroin solutions of the reaction mixture
on Florisil was the method employed.

The melting point of 14H-dibenzo[a,c]phenothiazine was found to
vary due to differences in the method and rate of heating of a sample
of the compound. The highest melting point was obtained when the melt-
ing point block was preheated to a temperature of ca. 150-155° before
introduction of the sample.

Unlike the thionation of other amines, in which mild conditions
would yield a large amount of unchanged amine while more vigorous con-
ditions produced tars as the main products, the thionation of N-phenyl-
9-phenanthrylamine did not respond markedly to changes in the time and
temperature of reaction. In general the reaction produced unchanged
N-phenyl-9-phenanthrylamine, tars and a small amount of 14H-dibenzo[a,c]-
phenothiazine. The tars were probably polymeric aryl sulfides formed by

intermolecular thionation. Possibilities also exist for occurrence of intramolecular thionation to produce compounds (III-2, III-3) other than the desired material.
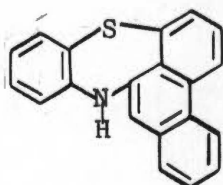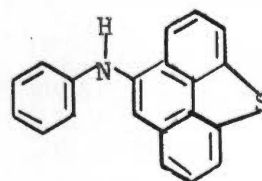
III-2   III-3

The designation of the structure of the thionation product as that corresponding to 14H-dibenzo[a,c] phenothiazine is based on two lines of evidence. First, the thionation of N-phenyl-9-phenanthryl-amine would be expected to occur preferentially at the reactive 10-position of the phenanthrene nucleus, just as the 1-position of the naphthalene ring is the site of reaction in N-phenyl-2-naphthylamine and di-2-naphthylamine.

The second point involves the metalation of the compound, which is described in more detail in the latter part of this chapter. The product of the metalation and carbonation was the lactam of a mono-carboxylic acid derivative of a dibenzophenothiazine. The formation of such a compound is best explained by the metalation of a compound, the structure of which corresponds to 14H-dibenzo[a,c] phenothiazine.

C. Nitrogen Mustard Derivatives

1. 2-Chloroethyl Derivatives

A modification of the procedure of Gilman and Shirley[36] was found to be the most satisfactory method for the synthesis of the 2-chloroethyl

derivatives of the phenothiazines. 10-Lithiophenothiazine was formed by the reaction of n-butyllithium and phenothiazine in ether. The solution was then cooled in an ice bath and allowed to react with 2-chloroethyl p-toluenesulfonate for a few hours. The product was obtained by chromatographic separation of the reaction mixture on Alcoa grade F-20 alumina. Satisfactory yields (40-60 per cent) were obtained in the cases of phenothiazine, 7H-benzo[c]-, 14H-dibenzo[a,c]- and 14H-dibenzo[a,h]phenothiazine. However, 7-(2-chloroethyl)dibenzo[c,h]-phenothiazine was produced in yields of only 8-25 per cent. The best yields were obtained using ether as the solvent and the poorest came from experiments which employed tetrahydrofuran and an ether-benzene mixture. Addition of 7-lithiodibenzo[c,h]phenothiazine to the sulfonate ester instead of the normal addition of the sulfonate to the lithio compound did not improve the yield.

In 1958 Gilman and Gray[74] reported the results of the metalation and carbonation of dibenzofuran, dibenzothiophene and N-ethylcarbazole in ether, tetrahydrofuran and a mixture of ether and tetrahydrofuran. The best yields were obtained when the lithium reagent was prepared in ether and added to a tetrahydrofuran solution of the compound undergoing metalation. Although the metalations of Gilman and Gray involved the replacement of hydrogen attached to the carbon instead of nitrogen, it was thought that tetrahydrofuran offered possibilities for the preparation of N-(2-chloroethyl) derivatives of the phenothiazines. However, the use of tetrahydrofuran and an ether-tetrahydrofuran mixture resulted in yields of only 6-11 per cent of 10-(2-chloroethyl)phenothiazine and 7-(2-chloroethyl)dibenzo[c,h]phenothiazine.

Chromatographic separation of the reaction mixtures from the preparation of the N-(2-chloroethyl) derivatives of phenothiazine and 7H-dibenzo[c,h]phenothiazine yielded besides the desired product small amounts of compounds, the elemental analyses of which corresponded to 1,2-di-(10-phenothiazinyl)ethane (III-4) and 1,2-di-(7-dibenzo[c,h]pheno-thiazinyl)ethane (III-5) respectively.
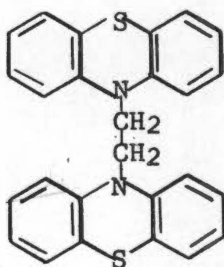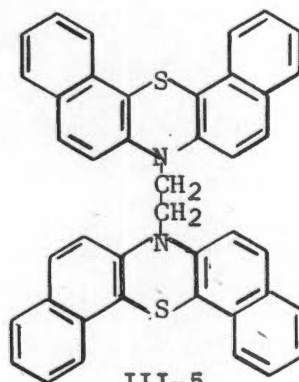
III-4   III-5

Spectral evidence supports the postulation of the structure III-4. A strong band in the region of 6.83-6.86 microns, the position at which methylene groups[75] absorb, appears in the infrared spectra of 10-methyl-and 10-(2-chloroethyl)phenothiazine and in the spectrum of the unknown compound. The ultraviolet absorption spectra of 10-methylphenothiazine and the unknown compound contain three bands, the positions of which are identical.

The products (III-6) were probably formed from the N-(2-chloro-ethyl) derivative and unreacted N-lithio compound as shown below.

41

III-6   + LiCl
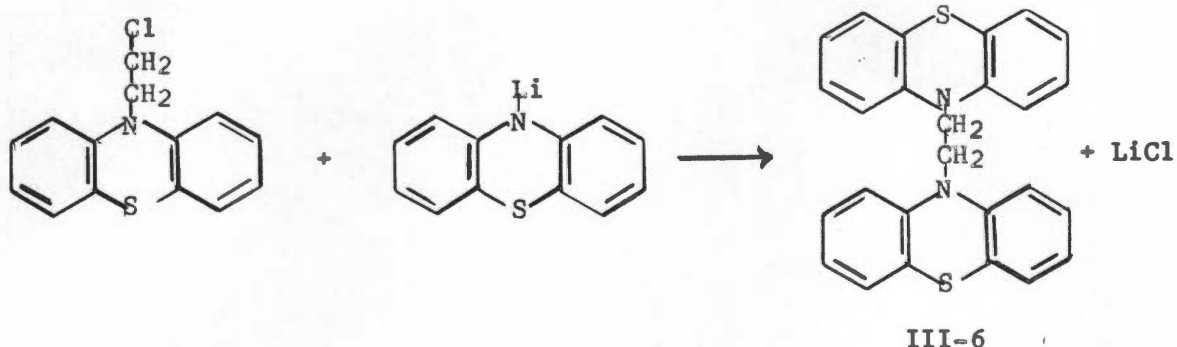
The investigations of Shirley and Liu[71] had shown that the 1-
and 12-positions of 12H-benzo[a]phenothiazine could be bridged by a
carbonyl group. They heated 1-carboxy-12H-benzo[a]phenothiazine and
formed the lactam, 12-oxo-12H-isoindolo[2,1,7 - m,n,a]phenothiazine
(III-7).
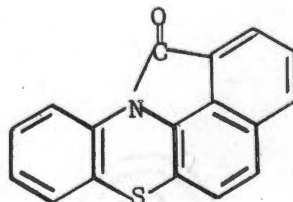
III-7

In the course of the metalation of 12H-benzo[a]phenothiazine with
two equivalents of n-butyllithium, the hydrogens at positions 1 and 12
are displaced by lithium.[71] Therefore, it was thought that reaction of
12H-benzo[a]phenothiazine with two equivalents of n-butyllithium fol-
lowed by the reaction of the metalation complex with 2-chloroethyl p-
toluenesulfonate would result in the bridging of the 1- and 12-positions
by an ethylene group (III-8). One reaction in which the molar ratio of
12H-benzo[a]phenothiazine:n-butyllithium:2-chloroethyl p-toluenesulfonate
was 1:2:1 and two reactions in which the ratio was 1:3:2 yielded 12-(2-
chloroethyl)benzo[a]phenothiazine as the only product which could be

III-8
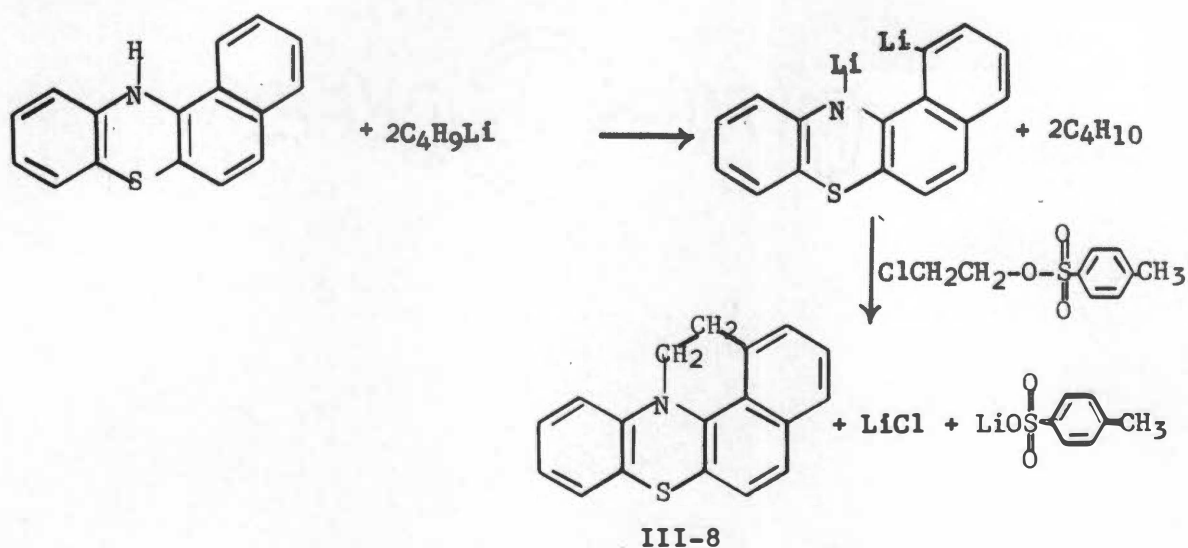
isolated and identified. The two reactions, the ratios of which were 1:3:2, resulted in the formation of the 12-(2-chloroethyl) derivative, m.p. 100-101.5°, in yields of 24 and 44 per cent.

Although the attempt to bridge the 1- and 12-positions was not successful, it is significant to note the formation of the 2-chloroethyl derivative in 44 per cent yield in the presence of an excess of n-butyl-lithium. Normally, N-substituted derivatives of the phenothiazines are formed by the reaction of 1 mole of the phenothiazine with 1 mole of n-butyllithium followed by the addition of 1 mole of alkyl halide or sul-fonate ester. The fact that the 2-chloroethyl p-toluenesulfonate reacted at the 12-position in preference to the 1-position was not expected on the basis of the relative reactivities with n-butyllithium of the hydrogen atoms attached to the two positions. Considering the metalation as an acid-base reaction, the 12-H is more acidic than the 1-H, since reaction is observed to occur first at the nitrogen atom. Therefore, the conjugate base formed by displacement of the hydrogen attached to carbon should be

the stronger than that formed by removal of hydrogen from nitrogen.

2. Reactions with Diethanolamine

The N-{2-[bis-(2-hydroxyethyl)amino]ethyl}derivatives of the phenothiazines were obtained in yields of approximately 80 per cent by the reaction of the N-(2-chloroethyl) derivative with a large excess of diethanolamine at 130-140° for 40 hours. The product of the reaction
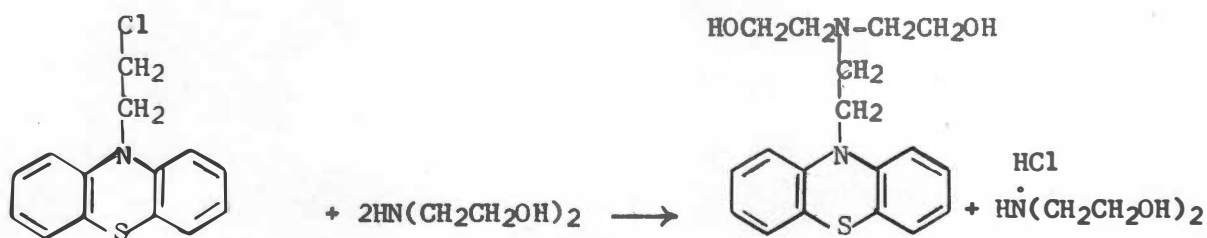
was isolated by extraction with benzene of an aqueous suspension of the reaction mixture followed by chromatographic separation of the benzene solution on Florisil. In each case the hydroxy compound was strongly adsorbed by the Florisil. The unchanged N-(2-chloroethyl) compound was removed by elution of the column with benzene, after which the desired product was brought down with a 1:9 mixture of acetone and benzene. In the cases of phenothiazine and 7H-benzo[c]phenothiazine, the product was an oil, but 7H-dibenzo[c,h]- and 14H-dibenzo[a,h]phenothiazine yielded solid derivatives.

3. N-{2-[Bis-(2-chloroethyl)amino]ethyl} Derivatives

Conversion of the hydroxy compounds to the nitrogen mustard derivatives (III-9) was accomplished in yields of 50-65 per cent by the action of phosphorus oxychloride, which also served as the solvent for the reaction. The procedure previously had been described by Fletcher and

III-9
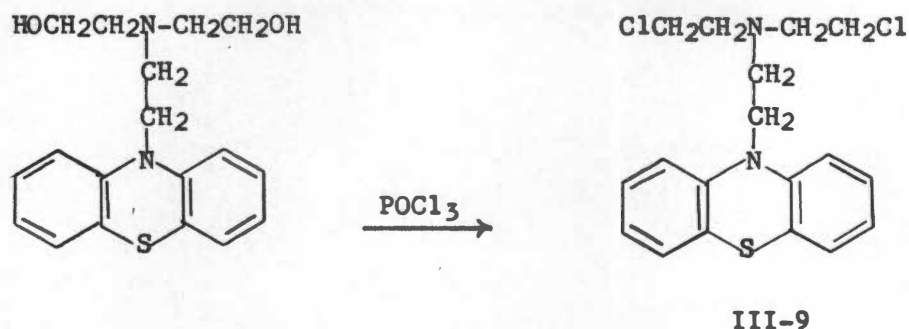
Wetzel[76] for the synthesis of nitrogen mustard derivatives of fluorene.

It was noted in the case of phenothiazine that if the temperature of the reaction during the mixing of the hydroxy compound and phosphorus oxychloride was allowed to reach the boiling point of phosphorus oxychloride, the yield of the nitrogen mustard was low (11 per cent). The investigations of Gerrard[77] had shown that n-butyl chloride, produced by the action of phosphorus oxychloride or phosphorus trichloride on n-butyl alcohol, arose from the interaction of hydrogen chloride with the intermediate alkyl phosphate or phosphite esters.

$$POCl_3 + 3BuOH + 3C_6H_5N \longrightarrow PO(OBu)_3 + 3C_6H_5N, HCl$$

Therefore, if a similar mechanism were involved in the formation of the nitrogen mustard with the initial formation of a phosphate ester and hydrogen chloride, it seems likely that part of the hydrogen chloride would be expelled by the boiling phosphorus oxychloride. Insufficient hydrogen chloride would then be available for reaction with the ester resulting in a decreased yield of the mustard derivative.

Gerrard[77] found that the yield of n-butyl chloride from the reaction of n-butyl alcohol and phosphorus trichloride was improved by agitating the reaction mixture with a stream of hydrogen chloride. The use of excess hydrogen chloride on the reaction of phosphorus oxychloride

with alcohols was not reported, but a similar improvement in the yield of halide would probably be observed.

An attempt to replace the hydroxyl group with a chlorine atom by the action of thionyl chloride in pyridine was unsuccessful. The reaction yielded a brown tar which was insoluble in acetone, ethanol, ether, benzene, tetrahydrofuran, chloroform, pyridine and dimethylformamide. Since the formation of alkyl chlorides by the reaction of thionyl chloride with a hydroxy compound has been shown to proceed through a dialkyl sulfite,[78] it is possible that the tar resulted from the formation of a polymeric sulfite ester (III-10).
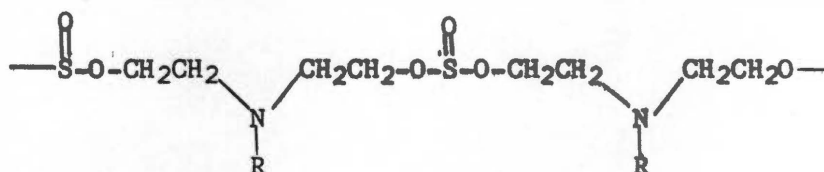
III-10

The nitrogen mustard was also formed by the reaction of phosphorus pentachloride with the N-{2-[bis-(2-hydroxyethyl)amino]ethyl} derivative in chloroform. Yields of the nitrogen mustard were not satisfactory with this reagent.

4. Hydrochloride Derivatives

The hydrochloride derivatives of the nitrogen mustards and the hydroxy intermediates were formed by passing dry hydrogen chloride through an alcoholic solution of the amine. The product was precipitated with ether.

Most of the hydrochlorides presented problems in purification, since recrystallization from an ethanol-ether mixture sometimes lowered rather than improved the melting point. The solvent appeared to be de- protonating the salt, a phenomenon which could be accounted for by the low basicity of the negatively substituted nitrogen atom. This problem was overcome by dissolving the hydrochloride in ethanol and then passing hydrogen chloride through the solution to insure the presence of excess hydrogen chloride.

The melting points of the salts varied with the temperature of introduction of the sample into the melting apparatus and with the rate of heating. In most cases a sample in an evacuated capillary gave a sharper melting point than one at atmospheric pressure.

D. Metalation of the Dibenzophenothiazines
with n-Butyllithium

1. 7H-Dibenzo[c,h]phenothiazine

The metalation of 7H-dibenzo[c,h]phenothiazine (III-11) was expected to occur in the 6-position. Gilman, Shirley and Van Ess[65] had shown that

III-11   III-12   III-13
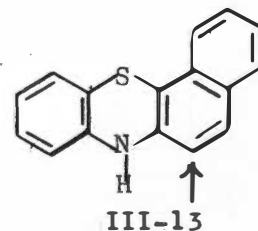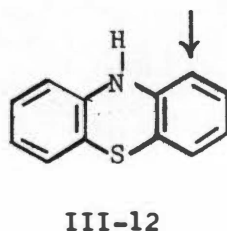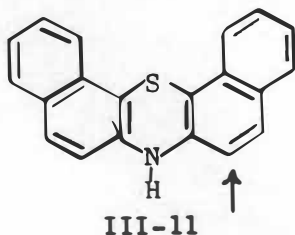
the 1-position was the reaction site in the metalation of phenothiazine (III-12). The metalation of 7H-benzo[c]phenothiazine (III-13) was

observed by Tatum[45] to take place in the naphthalene ring adjacent to nitrogen. Since 7H-dibenzo[c,h]phenothiazine contains two naphthalene ring positions similar to the reaction site in 7H-benzo[c]phenothiazine, the position of metalation of the dibenzo type could be predicted with confidence.

A monocarboxylic acid, m.p. 311-312° in vacuo, was obtained in 77 per cent yield by the reaction of 0.048 mole of n-butyllithium (hexane solution) and 0.020 mole of 7H-dibenzo[c,h]phenothiazine in anhydrous ether followed by carbonation of the metalation product with excess solid carbon dioxide. The methyl ester of the acid was prepared by the action of diazomethane.

Three attempts to desulfurize the acid with Raney nickel were unsuccessful. In each attempt the majority of the material isolated from the reaction was unchanged acid. Also isolated was a small amount of a material, the infrared spectrum of which exhibited a less intense carbonyl band than that of the original acid.

Desulfurization of the ester, 6-carbomethoxy-7H-dibenzo[c,h]-phenothiazine (III-14) with Raney nickel in ethanol was accomplished in

III-14
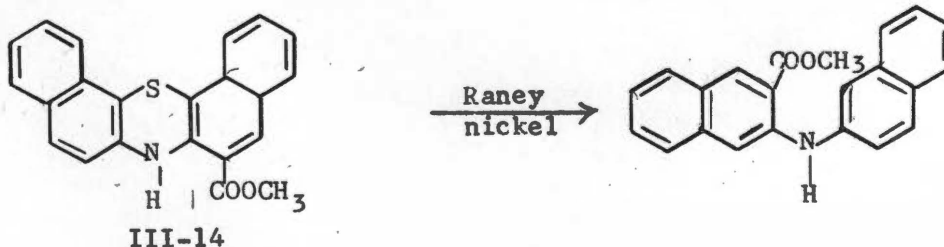
66 per cent yield. The desulfurization product, 3-carbomethoxy-2,2'-dinaphthylamine, was cyclized to dibenz[a,i]acridone (III-15) with polyphosphoric acid. The same compound had been reported previously by

III-15
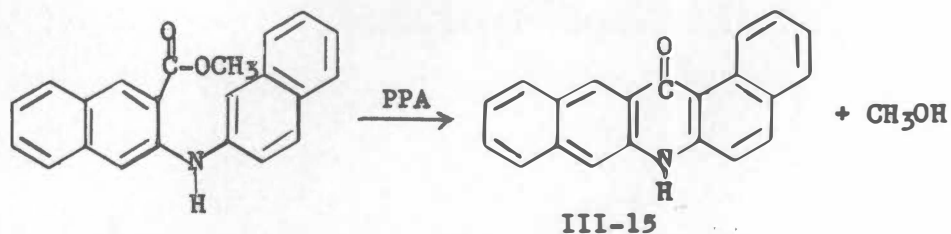
Strohback[79] and by Cymerman-Craig and Loder.[80] Thus the formation of dibenz[a,i]acridone proved that the structure of the acid formed by metalation and carbonation corresponded to 6-carboxy-7H-dibenzo[c,h]-phenothiazine.

2. 14H-Dibenzo[a,h]phenothiazine

The metalation of 14H-dibenzo[a,h]phenothiazine (III-16) provided an opportunity to compare the relative reactivities of the positions shown to be metalated preferentially in 12H-benzo[a]- and 7H-benzo[c]-phenothiazine. The 1-position of 14H-dibenzo[a,h]phenothiazine corresponds to the 1-position of 12H-benzo[a]phenothiazine (III-17), shown by Shirley and Liu[71] to be the metalation site of the benzo compound. Tatum[45] found that metalation of 7H-benzo[c]phenothiazine (III-18)

III-16   III-17   III-18
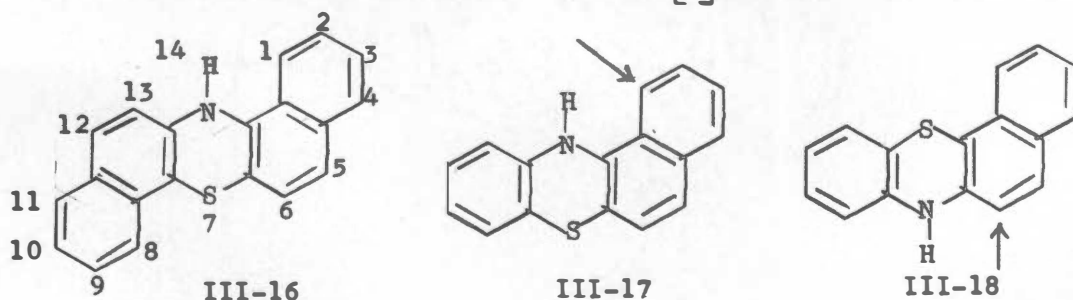
occurred at the 6-position, which in 14H-dibenzo[a,h]phenothiazine corresponds to the 13-position.

The reaction of 0.015 mole of n-butyllithium with 0.005 mole of 14H-dibenzo[a,h]phenothiazine followed by carbonation with excess carbon

dioxide resulted in the formation of a monocarboxylic acid derivative
in 80 per cent yield. The lactam (III-19) of the acid was formed by
heating a sample of the acid for 8 minutes at 190-210°. The elemental

III-19
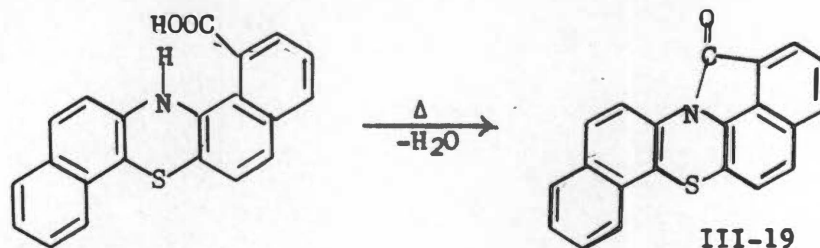
analysis of the product corresponded to the formula $C_{21}H_{12}NOS$, indicating
that the components of water had been eliminated from the carboxylic
acid. The carbonyl stretching band in the infrared spectrum of the lactam
appeared at 5.86 microns, shifting from the wavelength of 6.01 microns
observed in the spectrum of the acid.

The formation of the lactam is considered to be proof that the
metalation occurred in the 1-position. Had the metalation occurred in
the 13-position, the formation of a β-lactam would be necessary to explain
the elimination of water and the corresponding change in composition.
Such a lactam was not observed to form in the cases of 6-carboxy-7H-
dibenzo[c,h]phenothiazine or 6-carboxy-7H-benzo[c]phenothiazine.[45]

Shirley and Liu[71] proved the structure of the lactam of 1-
carboxy-12H-benzo[a]phenothiazine by desulfurization (see reaction II-
35, page 32) of the compound with Raney nickel to N-phenylnaphthastyril,
which was identical with a sample synthesized by an independent route.
A shift in the carbonyl stretching band from 6.01 microns for the acid
to 5.90 microns for the lactam was observed by these workers.

An unusual reaction was found to take place in an attempted preparation of the methyl ester of 1-carboxy-14H-dibenzo[a,h]phenothiazine using diazomethane. The lactam of the acid rather than the ester was isolated from the reaction mixture. The same transformation could not be effected in the ether-tetrahydrofuran reaction solvent in the absence of diazomethane. The mechanism of the reaction of diazomethane with carboxylic acids has apparently not been elucidated but has been postulated[81] to proceed by a 1,3 addition (III-20) in analogy to

III-20

the known formation of 5-membered rings (III-21) by the addition of

III-21

aliphatic diazo compounds to carbon-carbon multiple bonds.

A possible route for the lactam formation involves a cyclic transition state leading to the production of the intermediate (III-22) shown below.

III-22

The intermediate would be expected to lose methyl alcohol in a facile manner to form the lactam.

The study was extended to determine the effect of diazomethane on 1-carboxy-12H-benzo[a]phenothiazine,[71] which is structurally similar to 1-carboxy-14H-dibenzo[a,h]phenothiazine. It was found that the normal formation of the methyl ester (III-23) occurred in the case of the benzo compound.

III-23
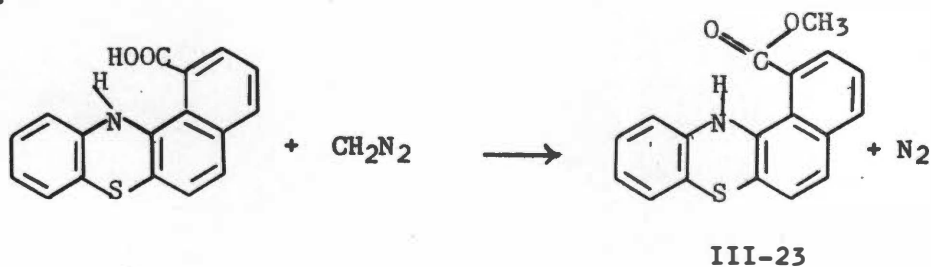
3. 14H-Dibenzo[a,c]phenothiazine

The metalation of 14H-dibenzo[a,c]phenothiazine was performed in a manner similar to that used for the preparation of the monocarboxylic acid derivatives of 7H-dibenzo[c,h]- and 14H-dibenzo[a,h]phenothiazine. However, the product, which was isolated in a yield of 84 per cent, was not the acid but the lactam (III-24) of the acid. The infrared spectrum
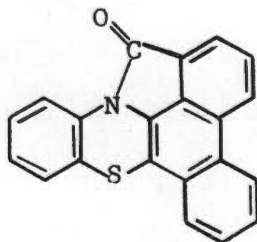
III-24

of the product revealed no hydrogen-bonded hydroxyl bands in the region 3-4 microns. These bands, characteristic of carboxylic acids, were

observed in the spectra of 1-carboxy-12H-benzo[a]phenothiazine and 1-carboxy-14H-dibenzo[a,h]phenothiazine but not in the lactams of these acids. The initial assumption of the presence of the lactam on the basis of spectral evidence was confirmed by the elemental analysis.

Since the product from metalation and carbonation was dissolved in water in order to separate it from unchanged 14H-dibenzo[a,c]-phenothiazine, the material must have been in the form of the lithium salt of the carboxylic acid. Therefore, the conversion to the lactam occurred either during or after the acidification of the aqueous solution.

4. Results of Metalation Studies

New indications of the specificity of the metalation reaction were obtained in the course of this research. The fact that yields of 77-84 per cent were produced in the metalations of single positions in the three dibenzophenothiazines is significant when the complexity of these polynuclear systems is considered. In each case metalation occurred at a position adjacent to nitrogen.

E. Miscellaneous Derivatives

The N-methyl and N-acetyl derivatives of 7H-dibenzo[c,h]phenothiazine were prepared in order to further characterize the dibenzo compound. The melting points of the two derivatives agreed with the values reported by Kym.[38,55]

The 14-methyl, 14-(2-chloroethyl), and 14-acetyl derivatives of 14H-dibenzo[a,c]phenothiazine prepared in this research have not been reported previously. It was found that the oxidation of the methyl compound with 30 per cent hydrogen peroxide in acetic acid could be controlled to yield either the sulfoxide or sulfone. The sulfoxide was produced by heating the mixture on the steam bath for a few minutes. Bands at 9.63 and 9.81 microns, characteristic of the sulfoxide group,[75] appeared in the infrared spectrum of 14-methyldibenzo[a,c]phenothiazine-9-oxide. A more vigorous oxidation using hydrogen peroxide in boiling acetic acid resulted in the formation of a very small amount of the sulfone. Identification was made on the basis of bands at 8.67 and 8.83 microns in the infrared spectrum of the product which are indicative of the sulfone group.[75]

F. Evaluation of Compounds as Anticancer Agents

All of the phenothiazines, the nitrogen mustard derivatives, and two of the hydroxy intermediates prepared in the course of this research were submitted to the Cancer Chemotherapy National Service Center for testing as described in Chapter I. At present the results are incomplete. The 10-{2-[bis-(2-chloroethyl)amino]ethyl} derivative of phenothiazine has shown activity in the Dunning leukemia test (mouse), but the only other mustard derivative for which a report was available, 10-(2-chloroethyl)phenothiazine, was not active against the same cancer. Four compounds, 10-{2-[bis-(2-hydroxyethyl)amino]ethyl}phenothiazine, 7-

{2-[bis-(2-hydroxyethyl)amino]ethyl}benzo[c]phenothiazine, 7H-dibenzo-
[c,h]phenothiazine and 7-methyldibenzo[c,h]phenothiazine were reported
to be inactive in the tests on sarcoma 180, carcinoma 755, and leukemia
1210.

G. The Ultraviolet Absorption Spectra of the Phenothiazines
and Diarylamines

A comparison of the ultraviolet spectra of phenothiazine, 7H-
benzo[c]phenothiazine and 7H-dibenzo[c,h]phenothiazine with the spectra
of the diarylamines from which they were synthesized indicates little
or no enhancement in conjugation due to cyclization to a polynuclear
aromatic system. Bathochromic shifts representing extended conjugation
are observed when one goes from the spectrum of di-2-naphthylamine to
that of 7H-dibenzo[c,h]phenothiazine, but the increments are only a few
millimicrons. The absorption bands in the spectrum of 7H-benzo[c]-
phenothiazine appear at slightly shorter wave lengths than the corre-
sponding bands in the spectrum of N-phenyl-2-naphthylamine.

The spectra of 7H-benzo[c]- and 7H-dibenzo[c,h]phenothiazine show
fewer and less intense bands than the spectra[83] of benz[a]- and dibenz-
[a,j]anthracene (III-25, III-26). Thus the conjugation present in the
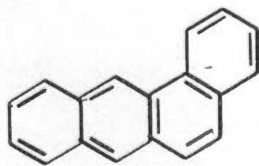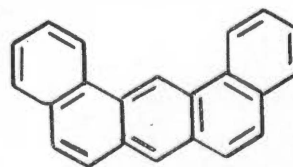
III-25   III-26

phenothiazine system is evidently not as strong as that of the anthra-
cenes. The spectrum[83] of dibenz[a,j]acridine (III-27) is also quite
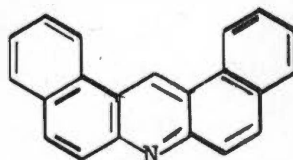
III-27

intense, representing strong conjugation in the polynuclear system.

The similarity of the spectra of the diarylamines and the pheno-
thiazines indicates that no appreciable conjugation is maintained
through the nitrogen and sulfur. The anthracene ring system is planar
allowing interaction between the π electrons through adjacent rings
with the resultant strong conjugation in the molecule. The spectral
evidence obtained in this research implies that the phenothiazine ring
system is probably "cupped," thus reducing the possibility for inter-
action of the π electrons between the two aromatic rings separated by
nitrogen and sulfur.

Table I contains a list of the maxima and log ϵ values of the
ultraviolet spectra of some of the phenothiazines and diarylamines.

TABLE I

THE MAXIMA OF THE ULTRAVIOLET SPECTRA OF SOME PHENOTHIAZINES AND DIARYLAMINES

Diphenylamine	λ	200			285		
	log ϵ	4.52			4.32		
Phenothiazine	λ	204	253			318	
	log ϵ	4.47	4.64			3.66	
N-Phenyl-2-naphthylamine	λ	221		273		310	348
	log ϵ	4.69		4.41		4.34	3.60
7H-Benzo[c]phenothiazine	λ	220		272.5			335
	log ϵ	4.65		4.55			3.40
Di-2-naphthylamine	λ	217.5		264	277.5	316	348
	log ϵ	4.87		4.60	4.34	4.42	3.98
7H-Dibenzo[c,h]phenothiazine	λ	223	257	267	281		357
	log ϵ	4.78	4.50	4.61	4.55		3.38

CHAPTER IV

EXPERIMENTAL*

A. Derivatives of Phenothiazine

1. 10-(2-Chloroethyl)phenothiazine

A modification of the procedure of Gilman and Shirley[36] was used in the preparation of this compound. The reaction vessel consisted of a 500-ml., 3-necked, round-bottomed flask equipped with a water-cooled condenser, a magnetic stirrer, and a dropping funnel. An atmosphere of dry nitrogen was maintained throughout the reaction.** To a slurry of 13.7 g. (0.069 mole) of phenothiazine*** in 250 ml. of sodium-dried ether was added 50.0 ml. of a 1.39 molar pentane-heptane solution of n-butyllithium⁺ in a period of 45 minutes. The bright yellow solution of 10-lithiophenothiazine was cooled in an ice bath, and a solution of 19.0 g. (0.081 mole) of 2-chloroethyl p-toluenesulfonate⁺⁺ in 50 ml. of anhydrous ether was added in 15 minutes. The mixture was stirred

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*Melting points were determined on a Mel-Temp melting point block. The elemental analyses were determined by the Weiler and Strauss Microanalytical Laboratory, Oxford, England, unless indicated otherwise.

**All reactions involving organometallic reagents were performed under a dry nitrogen atmosphere.

***Dow Chemical Company product.

⁺Foote Mineral Company product.

⁺⁺Eastman Organic Chemicals product.

for 1 hour at 0° and for 4 hours at room temperature. The lithium p-toluenesulfonate was removed by extraction with water. Benzene was added to the ethereal solution, and the ether was removed by evaporation. Sufficient ligroin (b.p. 100-115°) was added to produce a 7:3 mixture by volume of ligroin and benzene. The solution was divided into two equal parts, each of which was chromatographed on a 3.0 x 45-cm. column of Alcoa grade F-20 alumina. A 7:3 mixture of ligroin (b.p. 100-115°) and benzene eluted the product in the first fraction. A total of 11.9 g. (65.8 per cent) of colorless crystals, m.p. 94-96°, was obtained from the ligroin solution after removal of the benzene. Gilman and Shirley[36] reported a yield of 47 per cent and a melting point of 96-97°.

In a repetition of the procedure described above on a 0.10-mole scale, a small amount of a colorless, crystalline material, m.p. 205-207°, was isolated from the chromatographic separation of the reaction product mixture. The high melting compound followed the 10-(2-chloroethyl)phenothiazine from the column of alumina and was crystallized from a mixture of benzene and ligroin (b.p. 100-115°). The material appears to have the composition of 1,2-di-(10-phenothiazinyl)ethane.

Anal. Calcd. for $C_{26}H_{20}N_2S_2$: C, 73.55; H, 4.75; N, 6.70. Found: C, 73.63, 73.84; H, 4.71, 4.81; N, 6.56, 6.71.

The effect of varied reaction conditions on the yield of 10-(2-chloroethyl)phenothiazine are summarized in Table II.

TABLE II

EFFECT OF VARIED CONDITIONS ON THE YIELD
OF 10-(2-CHLOROETHYL)PHENOTHIAZINE

Solvent[a]	Temp. of Addition of 2-Chloroethyl p-Toluenesulfonate	Reaction Temperature	Reaction Time (Hrs.)	Yield (Per Cent)
Tetrahydrofuran	Ice Bath	Room Temp.	15	6.11
Tetrahydrofuran	Ice Bath	Reflux (65°)	19	9.17
Tetrahydrofuran-Ether (6:4)	Ice Bath	Ice Bath Room Temp.	0.5 20	7.64
Ether	Ice Bath	Ice Bath	0.5	37.2
Ether	Ice Bath	Ice Bath Room Temp.	1 4	65.8
Ether	Ice Bath	Ice Bath Room Temp.	1 4	59.5

[a]The tetrahydrofuran was dried by heating under reflux over sodium wire for several hours and then distilling from lithium aluminum hydride. Anhydrous ether was prepared by allowing the ether to stand over sodium wire.

2. <u>10-{2-[Bis-(2-hydroxyethyl)amino]ethyl}phenothiazine</u>[44]

Twelve grams of 10-(2-chloroethyl)phenothiazine was dissolved in 100 ml. of diethanolamine* in a 200-ml., round-bottomed flask. The mixture was stirred magnetically and heated to a temperature of 130-140° for a period of 40 hours. The solution was allowed to cool to room temperature. Water was added and the resulting suspension was extracted several times with 50-ml. portions of benzene. The combined benzene extracts were evaporated to a small volume and chromatographed on a 3.0 x 55-cm. column of Florisil.** Benzene eluted a small amount of 10-(2-chloroethyl)phenothiazine in the first fraction. The product then was eluted with a 1:9 mixture of acetone and benzene. After removal of the solvent by evaporation, 14.54 g. (96.2 per cent) of viscous, amber oil was obtained.

<u>Anal.</u>*** Calcd. for $C_{18}H_{22}N_2O_2S$: C, 65.42; H, 6.72; N, 8.47. Found: C, 65.52; H, 6.84; N, 8.42.

The amine was produced in 79 per cent yield in a repetition of the procedure described above. In a third preparation, reduction of the reaction time to 24 hours lowered the yield to 62 per cent.

3. <u>10-{2-[Bis-(2-hydroxyethyl)amino]ethyl}phenothiazine Hydrochloride</u>[44]

Dry hydrogen chloride was passed into a solution of 0.50 g. (0.00151 mole) of 10-{2-[bis-(2-hydroxyethyl)amino]ethyl}phenothiazine

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*Eastman Organic Chemicals product.

**Floridin Company product.

***Galbraith Laboratories, Knoxville, Tennessee.

in about 15 ml. of absolute ethanol. Evaporation of the ethanol yielded
an oil which crystallized when triturated with ether. The crude material
melted at 138-143°. After recrystallization from chloroform, pale pink
crystals, m.p. 143-144°, were obtained.

Anal. Calcd. for $C_{18}H_{23}ClN_2O_2S$: C, 58.93; H, 6.05; N, 7.63.
Found: C, 59.01, 58.85; H, 6.02, 5.87; N, 7.94, 7.72.

4. 10-{2-[Bis-(2-chloroethyl)amino]ethyl}phenothiazine[44]

The procedure described below is similar to that used by Fletcher
and Wetzel[76] for the preparation of nitrogen mustard derivatives of
fluorene. The reaction vessel consisted of a 100-ml., round-bottomed
flask equipped with an air-cooled condenser to which was attached a dry-
ing tube. Twenty-one milliliters of phosphorus oxychloride was added to
7.50 g. (0.0227 mole) of 10-{2-[bis-(2-hydroxyethyl)amino]ethyl}pheno-
thiazine which previously had been cooled in an ice bath. The mixture
was swirled and allowed to warm slowly so that the temperature of the
exothermic reaction, which occurred as the hydroxy compound was dissolved,
did not reach the boiling point of phosphorus oxychloride. After the
hydroxy compound had completely dissolved, the solution was heated on
the steam bath for 1 hour. Then the excess phosphorus oxychloride was
removed under diminished pressure on the steam bath, and the residue
was dissolved in acetone. The solution was poured over crushed ice,
an aqueous solution of sodium carbonate was added to neutralize the
hydrochloride, and the resulting mixture was extracted several times
with 50-ml. portions of benzene. The combined benzene extracts were
washed with water, concentrated to a small volume, and chromatographed

on a 3.0 x 40-cm. column of Florisil. Benzene eluted the product in the first fraction, which when evaporated to dryness yielded a pale yellow-green oil. The oil crystallized from ligroin (b.p. 66-75°) to yield 5.30 g. (63.5 per cent) of the colorless nitrogen mustard, m.p. 54.5-55.5°.

Anal. Calcd. for $C_{18}H_{20}Cl_2N_2S$: C, 58.85; H, 5.50; N, 7.62. Found: C, 58.99, 58.74; H, 5.44, 5.38; N, 7.53, 7.58.

A yield of 11.6 per cent was obtained when the initial reaction, during the mixing of the hydroxy compound and the phosphorus oxychloride, was allowed to become so vigorous that the boiling point of phosphorus oxychloride was reached. Repetition of the procedure described previously, in which control was exercised over the temperature of the reactants during mixing, resulted in a yield of 54 per cent.

5. 10-{2-[Bis-(2-chloroethyl)amino]ethyl}phenothiazine Hydrochloride[44]

Four grams (0.0109 mole) of the amine described above was dissolved in the minimum quantity of absolute ethanol. Dry hydrogen chloride was passed through the solution, which then was evaporated to a small volume. Ether was added until a slight, permanent cloudiness could be detected; after which the mixture was placed in the cold room for several hours. A yield of 3.34 g. (76.0 per cent) of colorless crystals, m.p. 128-132° in vacuo, was obtained. A preparation on a smaller scale gave a yield of 79.5 per cent of the hydrochloride, m.p. 126-131° at atmospheric pressure and 132-133° in vacuo.

Anal. Calcd. for $C_{18}H_{21}Cl_3N_2S$: C, 53.54; H, 5.24; N, 6.93. Found: C, 53.30, 53.30; H, 4.81, 4.99; N, 6.54, 6.59.

B. Derivatives of 7H-Benzo[c]phenothiazine

1. 7H-Benzo[c]phenothiazine

The procedure employed in the preparation of this compound was based on that of Tatum,[45] which was a modification of the procedure of Kehrman and Dardel.[40] The reactants, 21.9 g. (0.10 mole) of N-phenyl-2-naphthylamine, 6.42 g. (0.20 g. atom) of sulfur and 0.25 g. of iodine, were thoroughly mixed and placed in a Wood's metal bath at a temperature of 175-180° for 35 minutes. The warm melt was dissolved in acetone, and the resulting solution was filtered. About 150 ml. of benzene was added to the filtrate, and then the acetone and one-half of the added benzene were removed by evaporation. The benzene solution was allowed to remain in the cold room for several hours. The yellow-green product which crystallized from the solution was collected and dried on a Buchner funnel. The yield of crude 7H-benzo[c]phenothiazine was 17.73 g. (70.6 per cent), m.p. 174-176°. Tatum[45] reported a yield of 61 per cent of pure 7H-benzo[c]phenothiazine, m.p. 180-181°. Chromatographic purification of 3.0 g. of crude material on a 3.0 x 25-cm. column of Florisil (30-60 mesh) using benzene as the eluant yielded 2.8 g. of pure 7H-benzo[c]phenothiazine, m.p. 180.5-182°.

2. 7-(2-Chloroethyl)benzo[c]phenothiazine

Twenty-eight milliliters of a 1.48 molar solution of n-butyllithium in a pentane-heptane solvent was added in 20 minutes to a suspension of 10.00 g. (0.0398 mole) of 7H-benzo[c]phenothiazine in 700 ml. of anhydrous ether. The wine-red solution of 7-lithiobenzo[c]-phenothiazine was stirred (magnetic stirrer) under reflux for 45

minutes, after which it was cooled in an ice bath. A solution of 11.00 g. (0.0468 mole) of 2-chloroethyl p-toluenesulfonate in 50 ml. of anhydrous ether was added to the cold solution in a period of 10 minutes. The mixture was stirred for 45 minutes at 0° and for 20 hours at room temperature. Benzene and water were added to the reaction mixture and the benzene-ether layer was separated and concentrated to a small volume by evaporation. Sufficient ligroin (b.p. 100-115°) was added to produce a 7:3 mixture by volume of ligroin and benzene. Chromatographic separation of the solution on a 3.0 x 40-cm. column of Alcoa grade F-20 alumina, using a 7:3 mixture of ligroin (b.p. 100-115°) and benzene as the eluant, yielded 6.12 g. (49.1 per cent) of bright yellow crystals, m.p. 151-152°.

Anal. Calcd. for $C_{18}H_{14}ClNS$: C, 69.33; H, 4.53; N, 4.49. Found: C, 69.49, 69.85; H, 4.35, 4.81; N, 4.51, 4.22.

A yield of 23.9 per cent was obtained when the reaction above was repeated using anhydrous benzene as the solvent.

3. 7-{2-[Bis-(2-hydroxyethyl)amino]ethyl}benzo[c]phenothiazine

A mixture of 7.26 g. (0.0233 mole) of 7-(2-chloroethyl)benzo[c]-phenothiazine and 100 ml. of diethanolamine was stirred (magnetic stirrer) for 60 hours at a temperature of 135-140°. A cloudy, yellow suspension was present throughout the course of the reaction. The addition of water to the cooled mixture produced an aqueous suspension which was extracted several times with benzene. The combined benzene extracts were evaporated to a small volume and chromatographed on a 3.0 x 45-cm. column of Florisil. Benzene eluted a small quantity of unchanged 7-(2-chloro-

ethyl)benzo[c]phenothiazine. A 1:9 mixture of acetone and benzene was employed in the elution of the product, a viscous, yellow oil, which amounted to 9.20 g. after removal of the acetone and benzene.

Anal. Calcd. for $C_{22}H_{24}N_2O_2S$: C, 69.45; H, 6.36; N, 7.36. Found: C, 69.18, 69.34; H, 6.27, 6.41; N, 6.83, 6.92.

The isolated oil represents a yield greater than 100 per cent indicating that some solvent may have remained in the product. It is evident, however, that the yield was high since conversion of a 2-g. sample of the oil to the hydrochloride resulted in a yield of 85 per cent of the pure salt.

When the condensation between the chloride and diethanolamine was allowed to proceed for only 22 hours at 135-140°, a yield of 64 per cent was obtained.

4. 7-{2-[Bis-(2-hydroxyethyl)amino]ethyl}benzo[c]phenothiazine Hydrochloride

Dry hydrogen chloride was passed into a solution of 0.46 g. (0.00121 mole) of 7-{2-[bis-(2-hydroxyethyl)amino]ethyl}benzo[c]phenothiazine in about 15 ml. of absolute ethanol. The solution was evaporated to a volume of about 5 ml., benzene was added, and the remainder of the ethanol was removed. When cooled, the benzene solution yielded 0.39 g. (77 per cent) of colorless crystals, m.p. 213-215°. After recrystallization from a mixture of ethanol and ether the hydrochloride melted at 213.5-214.5°.

Anal. Calcd. for $C_{22}H_{25}ClN_2O_2S$: C, 63.37; H, 6.05; N, 6.72. Found: C, 63.45, 63.18; H, 5.71, 5.98; N, 6.68, 6.59.

5. 7-{2-[Bis-(2-chloroethyl)amino]ethyl}benzo[c]phenothiazine

Twenty-one milliliters of phosphorus oxychloride was added to
7.05 g. (0.00185 mole) of 7-{2-[bis-(2-hydroxyethyl)amino]ethyl}benzo-
[c]phenothiazine which had been cooled in an ice bath. The temperature
of the mixture was allowed to increase gradually. After all of the
hydroxy compound had dissolved in the phosphorus oxychloride, the dark
solution was heated on the steam bath for 75 minutes. Excess phos-
phorus oxychloride was removed under reduced pressure (water aspirator).
The residue was dissolved in acetone and the resulting solution was
poured over ice. After neutralization with an aqueous solution of
sodium carbonate, the mixture was extracted several times with benzene.
The benzene extracts were combined, washed with water, and concentrated
to a small volume. Chromatographic separation of the solution on a
3.0 x 45-cm. column of Florisil yielded 4.43 g. (57.3 per cent) of
viscous, yellow oil which was eluted with benzene in the first fraction.

Anal. Calcd. for $C_{22}H_{22}Cl_2N_2S$: C, 63.31; H, 5.32; N, 6.71.
Found: C, 64.36, 64.53; H, 5.11, 5.27; N, 6.46, 6.65.

Although the difference in the calculated and determined values
for the percentage of carbon is rather large, the hydrochloride deriva-
tive was shown to have the proper composition.

On a smaller scale the conversion was accomplished in a yield
of 65 per cent.

6. 7-{2-[Bis-(2-chloroethyl)amino]ethyl}benzo[c]phenothiazine Hydro-
chloride

A solution of 4.43 g. (0.0106 mole) of the amine in the minimum
quantity of absolute ethanol was treated with dry hydrogen chloride.
The volume of the solution was reduced by evaporation, after which
ether was added until a permanent cloudiness was detected. The hydro-
chloride was allowed to crystallize slowly from the ether-ethanol mix-
ture in the cold room. A yield of 3.50 g. (72.6 per cent) of pale
yellow crystals, m.p. 174.5-176.5° in vacuo, was obtained.

Anal. Calcd. for $C_{22}H_{23}Cl_3N_2S$: C, 58.22; H, 5.11; N, 6.17.
Found: C, 58.20, 58.16; H, 5.29, 5.05; N, 6.09, 6.21.

C. Derivatives and Metalation of 7H-Dibenzo[c,h]phenothiazine

1. 7H-Dibenzo[c,h]phenothiazine

a. Thionation of 2-naphthylamine. A modification of the procedure
of Knoevenagel[39] was employed in the preparation of this compound. A
mixture of 14.30 g. (0.10 mole) of 2-naphthylamine, 3.20 g. (0.10 g.
atom) of sulfur and 0.07 g. of iodine was heated for 3 hours at 195-
200° in a Glascol heater. The warm melt was dissolved in acetone and
the solution was filtered. About 100 ml. of benzene was added to the
filtrate and the acetone was removed by evaporation. The benzene solu-
tion was extracted three times with 50-ml. portions of 5 per cent hydro-
chloric acid and then was washed with water. The benzene layer was
concentrated to a small volume and placed in the cold room. The yield
of crude, yellow-green 7H-dibenzo[c,h]phenothiazine was 7.73 g. (51.7

per cent), m.p. 222-226° with decomposition. Four successive recrystal-
lizations from benzene of the crude material produced yellow needles,
m.p. 225-226° with decomposition. Recrystallization of this material
from ethanol and from acetone did not improve the melting point. Chroma-
tographic purification of 0.86 g. of crude material on a 1.5 x 38-cm.
column of Florisil (30-60 mesh) using benzene as the eluant yielded
0.65 g. of yellow needles, m.p. 224-225.5° with decomposition. It was
found that a sample of pure 7H-dibenzo[c,h]phenothiazine melted at
245-247° in vacuo with no decomposition.

The yield reported by Knoevenagel[39] was 80 per cent based on
crude material melting at 228°. After repeated recrystallization from
benzene he obtained pure 7H-dibenzo[c,h]phenothiazine, m.p. 236°.

b. Thionation of di-2-naphthylamine. The procedure used in the
preparation of this compound is essentially that of Knoevenagel,[39]
modified only in the temperature and time of reaction. A mixture of
13.5 g. (0.05 mole) of di-2-naphthylamine,* 3.2 g. (0.10 g. atom) of
sulfur, and 0.13 g. of iodine was placed in a 250-ml., round-bottomed
flask and was heated by a Glascol heater. At a temperature of about
140° the reactants momentarily formed a melt and then immediately re-
solidified. The temperature was increased to 165° but no observable
further change occurred. The cooled product was dissolved in the mini-
mum quantity of acetone and the solution was filtered. Benzene was
added and the acetone was removed by evaporation. The product crystal-

*Aldrich Chemical Company, Inc.

lized from benzene to yield 13.33 g. (89.1 per cent) of yellow needles, m.p. 222-225°. After two recrystallizations from benzene, a sample of the compound melted at 228-230° with decomposition and at 245-246° in vacuo with no decomposition. A yield of 79 per cent was obtained when the procedure above was repeated. Knoevenagel[39] reported a yield of 80-90 per cent and a melting point of 230° after one recrystallization from benzene. A value of 222-223° was recorded by Fang, Liu and Sah[57] in 1937 for the product of the thionation of di-2-naphthylamine.

2. 7-Acetyldibenzo[c,h]phenothiazine

A procedure similar to the one described below was used by Kehrman, Gressley, Chiffere and Ramm[61] for the preparation of 14-acetyldibenzo-[a,j]phenothiazine. A mixture of 0.25 g. of 7H-dibenzo[c,h]phenothiazine, 1.25 ml. of acetic anhydride and 0.25 g. of zinc chloride was allowed to react at room temperature for 1 hour. Ice water was added and the mixture was stirred for about 2 hours in order to decompose the excess acetic anhydride. The gray solid which was precipitated by the addition of water was removed by filtration, washed with water, and dried. The crude product melted at 190-195°. After five recrystallizations from ethanol, colorless crystals, m.p. 210.5-212°, were obtained. Kehrman and Christopoulos[56] reported a melting point of 208° and Kym[55] found the melting point to be 211°.

3. 7-Methyldibenzo[c,h]phenothiazine

To a slurry of 6.48 g. (0.0216 mole) of 7H-dibenzo[c,h]pheno-thiazine in 150 ml. of anhydrous benzene was added 15.0 ml. (0.0216

mole) of a pentane-heptane solution of n-butyllithium in a period of
10 minutes. The orange suspension which formed was stirred under re-
flux for 1 hour, after which a solution of 4.05 g. (0.0432 mole) of
methyl p-toluenesulfonate in 10 ml. of anhydrous benzene was added in
a 15-minute period. The mixture was stirred under reflux for 4 hours
and then was allowed to cool to room temperature. The yellow solid
which had precipitated during the reaction was removed by filtration.
After thorough washing with water, a bright yellow solid remained,
which when dried amounted to 4.20 g. (62.0 per cent), m.p. 280-281°.

After recrystallization from benzene, the yellow needles melted
at 280.5-281.5°. The material melted sharply at 303° in vacuo. Kym[38]
reported a melting point of 284-285°.

4. 7-(2-Chloroethyl)dibenzo[c,h]phenothiazine

Fifteen grams (0.050 mole) of 7H-dibenzo[c,h]phenothiazine was
suspended in 700 ml. of anhydrous ether. The addition of 37.0 ml. of
a 1.40 molar hexane solution of n-butyllithium produced a bright red
suspension of 7-lithiodibenzo[c,h]phenothiazine which then was stirred
for 1 hour at room temperature. The red suspension was cooled in an
ice bath and a solution of 14.0 g. (0.0596 mole) of 2-chloroethyl p-
toluenesulfonate in 50 ml. of anhydrous ether was added in 5 minutes.
The mixture was stirred for 1 hour at room temperature and for 20 hours
under gentle reflux. Then benzene and water were added to the reaction
mixture. The benzene-ether layer was separated, filtered, and concen-
trated to a small volume by evaporation. Sufficient ligroin (b.p. 100-
115°) was added to produce a 7:3 mixture by volume of ligroin and

benzene. The solution was chromatographed on Alcoa grade F-20 alumina.
A 7:3 mixture of ligroin (b.p. 100-115°) and benzene eluted the product
in the first fraction, which when evaporated to a small volume yielded
4.45 g. (25.2 per cent) of pale yellow needles, m.p. 184-185°.

Anal. Calcd. for $C_{22}H_{16}ClNS$: C, 73.02; H, 4.46; N, 3.87.
Found: C, 73.02, 73.24; H, 4.53, 4.76; N, 4.00, 4.08.

From the last fraction of the chromatographic separation of the
reaction mixture was isolated a red crystalline material, m.p. 240-
240.5°. A large volume of benzene was required to elute the compound.
The elemental analysis indicates that the structure of the compound
corresponds to 1,2-di-(7-dibenzo[c,h]phenothiazinyl)ethane.

Anal. Calcd. for $C_{42}H_{28}N_2S_2$: C, 80.75; H, 4.52; N, 4.48.
Found: C, 80.33, 80.45; H, 4.53, 4.41; N, 4.18, 4.45.

A summary of a number of preparations of 7-(2-chloroethyl)dibenzo-
[c,h]phenothiazine is contained in Table III.

5. 7-{2-[Bis-(2-hydroxyethyl)amino]ethyl}dibenzo[c,h]phenothiazine

A mixture of 5.45 g.(0.0151 mole) of 7-(2-chloroethyl)dibenzo-
[c,h]phenothiazine and 120 ml. of diethanolamine was stirred at a
temperature of 130-135° for 40 hours. The yellow solution was allowed
to cool to room temperature, after which water was added. The product
was removed from the aqueous suspension by several extractions with
50-ml. portions of benzene. The combined benzene extracts were concen-
trated to a small volume and chromatographed on a 1.8 x 63-cm. column
of Florisil. Benzene removed a small amount of unchanged 7-(2-chloro-
ethyl)dibenzo[c,h]phenothiazine. A 1:9 mixture of acetone and benzene

TABLE III

EFFECT OF VARIED CONDITIONS ON THE YIELD OF 7-(2-CHLOROETHYL)DIBENZO[c,h]PHENOTHIAZINE

Solvent	Order of Addition	Temperature of Addition	Reaction Temperature	Reaction Time (Hrs.)	Yield (Per Cent)
Benzene	7-Lithio DBP[a] to Sulfonate	Room Temperature	Reflux	15	20.5
Ether-Benzene	7-Lithio DBP to Sulfonate	Reflux (80°)	Reflux (80°)	20	8.3
Tetrahydrofuran	Sulfonate to 7-Lithio DBP	Ice Bath	Room Temp. Reflux	17 4	11.2
Ether	Sulfonate to 7-Lithio DBP	Ice Bath	Room Temp.	91	25.4
Ether	Sulfonate to 7-Lithio DBP	Ice Bath	Reflux	20	25.2

[a]The abbreviation "DBP" refers to dibenzo[c,h]phenothiazine.

eluted the product, which crystallized from benzene after removal of the acetone. A yield of 5.44 g. (84.0 per cent) of yellow crystals, m.p. 159-160°, was obtained.

<u>Anal</u>. Calcd. for $C_{26}H_{26}N_2O_2S$: C, 72.54, H, 6.08; N, 6.51. Found: C, 72.70, 72.46; H, 5.92, 6.13; N, 6.49, 6.37.

6. <u>7-{2-[Bis-(2-hydroxyethyl)amino]ethyl}dibenzo[c,h]phenothiazine</u>
   <u>Hydrochloride</u>

Dry hydrogen chloride was passed through a solution of 0.50 g. (0.00116 mole) of the amine in the minimum quantity of absolute ethanol. A pale yellow crystalline material immediately precipitated from the solution to yield 0.51 g. (94.5 per cent) of the hydrochloride, m.p. 256-261° at atmospheric pressure and 256-257° <u>in vacuo</u>.

<u>Anal</u>. Calcd. for $C_{26}H_{27}ClN_2O_2S$: C, 66.87; H, 5.82; N, 6.00. Found: C, 66.17, 66.77; H, 5.85, 5.82; N, 6.01, 6.16.

7. <u>7-{2-[Bis-(2-chloroethyl)amino]ethyl}dibenzo[c,h]phenothiazine</u>

A solution of 5.44 g. (0.0127 mole) of 7-{2-[bis-(2-hydroxy-ethyl)amino]ethyl}dibenzo[c,h]phenothiazine in 15.0 ml. of phosphorus oxychloride was heated on the steam bath for 1.5 hours. After removal of the excess phosphorus oxychloride under reduced pressure, the dark mass which remained was dissolved in acetone and the solution was poured over crushed ice. The acetone-water mixture was neutralized with aqueous sodium carbonate and then was extracted three times with benzene. The combined benzene extracts were washed with water, evaporated to a small volume, and chromatographed on a Florisil column.

Benzene eluted the product in the first fraction. Ligroin (b.p. 100-115°) was added to this fraction and the benzene was removed by evaporation. Crystallization from ligroin yielded 3.94 g. (66.7 per cent) of bright yellow needles, m.p. 117-118°.

Anal. Calcd. for $C_{26}H_{24}Cl_2N_2S$: C, 66.80; H, 5.18; N, 5.99. Found: C, 66.69, 67.01; H, 5.21, 5.03; N, 5.95, 5.81.

8. 7-{2-[Bis-(2-chloroethyl)amino]ethyl}dibenzo[c,h]phenothiazine Hydrochloride

Dry hydrogen chloride was passed through a solution of 4.10 g. (0.00878 mole) of the amine in the minimum quantity of absolute ethanol. Ether was added until a permanent cloudiness was produced and the mixture was placed in the cold room for several hours. A yield of 3.89 g. (88.2 per cent) of colorless crystals, m.p. 190-199° in vacuo, was collected. Recrystallization of the material from a mixture of ethanol and ether, into which was passed dry hydrogen chloride, gave 3.30 g. of the hydrochloride, m.p. 196-197.5° in vacuo.

Anal. Calcd. for $C_{26}H_{25}Cl_3N_2S$: C, 61.98; H, 5.01; N, 5.56. Found: C, 62.06, 62.05; H, 5.15, 4.91; N, 5.66, 5.48.

9. Metalation of 7H-Dibenzo[c,h]phenothiazine

a. 6-Carboxy-7H-dibenzo[c,h]phenothiazine. Thirty-two milliliters (0.048 mole) of a hexane solution of n-butyllithium was added in a period of 10 minutes to a slurry of 6.00 g. (0.020 mole) of 7H-dibenzo[c,h]-phenothiazine in 200 ml. of anhydrous ether. The resulting red suspension was stirred under reflux for 4.5 hours. The mixture was cooled in a

solid carbon dioxide-acetone bath, then carbonated with excess crushed solid carbon dioxide, and allowed to warm gradually to room temperature. The lithium salt of the carboxylic acid was removed by extraction with water. The combined aqueous extracts were washed with ether and then acidified with 50 per cent hydrochloric acid. The maroon-colored precipitate was collected on a sintered glass suction funnel, washed with water, and dried overnight in a vacuum desiccator. A yield of 5.27 g. (76.6 per cent) of the crude acid, m.p. 311-312° in vacuo and 297-303° at atmospheric pressure, was obtained. The infrared spectrum* of the material revealed a carbonyl stretching band at 5.95 microns.

Anal. Calcd. for $C_{21}H_{13}NO_2S$: C, 73.50; H, 3.81; N, 4.07. Found: C, 73.40, 73.39; H, 4.26, 4.01; N, 4.11, 4.00.

Attempts to purify the acid by recrystallization and by chromatography were unsuccessful. The structure of the acid was shown to correspond to 6-carboxy-7H-dibenzo[c,h]phenothiazine by desulfurization of its methyl ester and subsequent formation of dibenz[a,i]acridone.

b. 6-Carbomethoxy-7H-dibenzo[c,h]phenothiazine. A solution of diazomethane (ca. 0.05 mole) in ether was prepared, according to the procedure of DeBoer and Backer,[84] from N-methyl-N-nitroso-p-toluene-sulfonamide. To the solution of diazomethane in 160 ml. of ether was added 2.30 g. (0.00670 mole) of 6-carboxy-7H-dibenzo[c,h]phenothiazine

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*Infrared spectra were determined on a Perkin-Elmer Model 137B Infracord using the potassium bromide disc technique (1 mg. of sample in 300 mg. of potassium bromide). The spectra are on file in the Department of Chemistry, The University of Tennessee.

which was dissolved in 90 ml. of freshly distilled tetrahydrofuran.

After the addition of the acid, the evolution of a gas was observed.

The dark red solution was placed under a hood overnight to allow the

solvent and the excess diazomethane to evaporate. During this inter-

val the color of the solution lightened to bright red. Ligroin (b.p.

66-75°) was added and the remainder of the ether and tetrahydrofuran

was removed on the steam bath. A yield of 2.14 g. (89.4 per cent)

of the bright orange ester, m.p. 178-181°, crystallized from the

ligroin solution.

Anal. Calcd. for $C_{22}H_{15}NO_2S$: C, 73.92; H, 4.23; N, 3.92.

Found: C, 73.70, 73.93; H, 4.29, 4.19; N, 3.66, 3.64.

c. Desulfurization of 6-carbomethoxy-7H-dibenzo[c,h]pheno-

thiazine with Raney nickel. Activated Raney nickel was prepared

according to the procedure of Covert and Adkins.[85] Although a number

of attempts to desulfurize the acid were unsuccessful, it was found

that the methyl ester reacted smoothly.

To a suspension of 1.73 g. (0.00484 mole) of 6-carbomethoxy-7H-

dibenzo[c,h]phenothiazine in 200 ml. of absolute ethanol was added

14 g. of freshly prepared Raney nickel catalyst. The mixture was

stirred (magnetic stirrer) under reflux for 2 hours, after which time

there was no visible evidence that a reaction had occurred. When an

additional 6 g. of the catalyst was added to the hot mixture, the color

was immediately changed from orange to yellow. The mixture was refluxed

for another 2 hours, after which the ethanol solution was decanted from
the catalyst and carefully filtered on a sintered glass suction funnel.
From the solution was obtained 1.04 g. (65.6 per cent) of yellow needles,
m.p. 122-123°.

Anal. Calcd. for $C_{22}H_{17}NO_2$: C, 80.70; H, 5.24; N, 4.27.
Found: C, 80.66, 80.32; H, 5.16, 5.16; N, 4.10, 4.44.

The structure of the desulfurization product was shown to corre-
spond to 3-carbomethoxy-2,2'-dinaphthylamine by cyclization with poly-
phosphoric acid to dibenz[a,i]acridone.

d. Dibenz[a,i]acridone. One-fourth of a gram (0.000764 mole)
of 3-carbomethoxy-2,2'-dinaphthylamine from the desulfurization of 6-
carbomethoxy-7H-dibenzo[c,h]phenothiazine was dissolved in 40 ml. of
polyphosphoric acid* with heating and stirring. The deep-red solution
was heated at a temperature of 120-125° for 1.5 hours. Addition of the
solution to water precipitated a brown solid which was removed by fil-
tration and washed with water. A solution of the material in acetone
was filtered, benzene was added to the filtrate, and the acetone was
removed by evaporation. Then the benzene solution was chromatographed
on a 1.5 x 25-cm. column of Florisil. The majority of the product was
eluted with benzene in the first fraction. A 1:9 mixture of acetone
and benzene eluted an additional small amount of the acridone. A yield
of 0.132 g. (58.4 per cent) of bright yellow-gold dibenz[a,i]acridone,
m.p. 372-374°, crystallized from a mixture of ligroin (b.p. 100-115°)

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*Victor Chemical Works.

and benzene. Strohbach[79] reports a melting point greater than 300°
and Cymerman-Craig and Loder[80] obtained golden-red plates, m.p. 372°.

    e. 3-Carboxy-2,2'-dinaphthylamine. A small sample of the methyl
ester from the desulfurization of 6-carbomethoxy-7H-dibenzo[c,h]pheno-
thiazine was dissolved in approximately 30 ml. of methanol. Three grams
of sodium hydroxide was dissolved in the yellow solution and the result-
ing mixture was refluxed on the steam bath for 1.5 hours. The solution
was poured into 100 ml. of water and the aqueous mixture was acidified
with 50 per cent hydrochloric acid. The yellow material which precipi-
tated was removed by filtration, washed with water and dried. After
one recrystallization of the solid from ligroin (b.p. 66-75°) and two
recrystallizations from ether, bright yellow needles, m.p. 229-230°,
were obtained.

    Anal. Calcd. for $C_{21}H_{15}NO_2$: C, 80.47; H, 4.82; N, 4.47.
Found: C, 80.28, 80.07; H, 4.79, 4.79; N, 4.46.

    D. Derivatives and Metalation of 14H-Dibenzo[a,h]phenothiazine

1. 14H-Dibenzo[a,h]phenothiazine

    Following the procedure of Waters[43] which was a modification of
an earlier method of Kehrman, Gressly, Chiffere and Ramm,[61] 50.00 g.
(0.1967 mole) of 1,2'-dinaphthylamine,[43] 12.60 g. (0.393 g. atom) of
sulfur and 0.50 g. of iodine were mixed thoroughly and heated by means
of a Glascol heater. The mixture melted at a temperature of about 100-
110°. The temperature was raised to 130-135° and maintained for 12

minutes, at which point the melt resolidified. The warm mass was
crushed and washed with acetone. The remaining solid was placed in a
Soxhlet extractor and extracted with benzene. The initial acetone
washings yielded 7.70 g. of unchanged amine. The benzene extracts
yielded 16.75 g. of pure product, m.p. 200-203° in vacuo, and 12.10 g.
of impure material, m.p. 170-180° in vacuo. The total yield was
28.85 g. (48.9 per cent). Waters[43] reported yields in the range 45-
56 per cent and a melting point of 194-197° in vacuo on unchromato-
graphed 14H-dibenzo[a,h]phenothiazine.

2. 14-(2-Chloroethyl)dibenzo[a,h]phenothiazine

To a slurry of 8.00 g. (0.0267 mole) of 14H-dibenzo[a,h]pheno-
thiazine in 450 ml. of anhydrous ether was added 22.0 ml. of a 1.50
molar hexane solution of n-butyllithium in a period of 10 minutes.
The resulting red solution was stirred (magnetic stirrer) for 2 hours,
after which it was cooled in an ice bath. A solution of 2-chloroethyl
p-toluenesulfonate in 50 ml. of anhydrous ether was added in 5 minutes
to the cool solution of 14-lithiodibenzo[a,h]phenothiazine. The mix-
ture was stirred for 1 hour at 0° and for 18 hours under reflux. The
reaction mixture was filtered and extracted with water to remove the
lithium p-toluenesulfonate. A 2:3 mixture of benzene and ligroin
(b.p. 100-115°) was added and the ether was removed by evaporation.
The resulting solution was chromatographed on two 1.7 x 70-cm. columns
of Florisil (60-100 mesh) using ligroin (b.p. 100-115°) as the eluant.
A yield of 3.80 g. (39.2 per cent) of yellow product, m.p. 146-148°,
crystallized from the first fraction. Two additional preparations, one

of which was stirred at room temperature for 42 hours and under reflux for 3 hours and the other at room temperature for 35 hours and under reflux for 1 hour, resulted in yields of about 40 per cent. The purest material which was obtained melted at 148-149°. Waters[43] reported a yield of 25.6 per cent and a melting point of 147-148°.

3. 14-{2-[Bis-(2-hydroxyethyl)amino]ethyl}dibenzo[a,h]phenothiazine

A mixture of 7.50 g. (0.0207 mole) of 14-(2-chloroethyl)dibenzo-[a,h]phenothiazine and 150 ml. of diethanolamine was stirred (magnetic stirrer) and heated at a temperature of 130-135° for 40 hours. Water was added to the reaction mixture and the resulting aqueous suspension was extracted several times with benzene. The combined benzene extracts were washed with water, concentrated to a small volume, and chromato-graphed on Florisil. Benzene brought down a small amount of unchanged 14-(2-chloroethyl)dibenzo[a,h]phenothiazine in the initial fraction. A 1:9 mixture of acetone and benzene eluted the product. The solvent was removed by evaporation and the residue was dissolved in a mixture of ligroin (b.p. 66-75°) and ether, from which 7.46 g. (83.7 per cent) of yellow crystals, m.p. 136-137°, was obtained.

Anal. Calcd. for $C_{26}H_{26}N_2O_2S$: C, 72.54; H, 6.08; N, 6.51. Found: C, 72.60, 72.42; H, 5.97, 6.05; N, 6.31, 6.45.

Repetition of the procedure above resulted in a yield of 81 per cent.

The compound exhibits the phenomenon of dimorphism, having a second crystalline form which melts at 107-108°. One recrystallization

from a mixture of ligroin and ether raised the melting point of the
lower melting form to 136-137°. The infrared spectra of the two forms
are identical.

4. 14-{2-[Bis-(2-hydroxyethyl)amino]ethyl}dibenzo[a,h]phenothiazine
    Hydrochloride

Dry hydrogen chloride was passed through a solution of 0.30 g.
(0.000698 mole) of the amine in anhydrous ether. The hydrochloride
precipitated immediately. A yield of 0.16 g. (49.1 per cent) of the
pale yellow material, m.p. 208-210° at atmospheric pressure and 211.5-
213° in vacuo was obtained.

Anal. Calcd. for $C_{26}H_{27}ClN_2O_2S$: C, 66.87; H, 5.82; N, 6.00.
Found: C, 66.73, 66.73; H, 6.05, 5.78; N, 6.03, 5.88.

5. 14-{2-[Bis-(2-chloroethyl)amino]ethyl}dibenzo[a,h]phenothiazine

Eleven and five-tenths milliliters of phosphorus oxychloride was
added to 3.80 g. (0.00884 mole) of 14-{2-[bis-(2-hydroxyethyl)amino]-
ethyl}dibenzo[a,h]phenothiazine in a 50-ml., round-bottomed flask which
was suspended in an ice bath. The hydroxy compound was dissolved
slowly in the phosphorus oxychloride by gradually increasing the temper-
ature of the mixture. The resulting dark green solution was heated on
the steam bath for 1.5 hours, after which the excess phosphorus oxy-
chloride was removed under reduced pressure. The residue was dissolved
in acetone and the solution was poured over crushed ice. The acetone-
water mixture was neutralized with a solution of sodium carbonate and
then was extracted with benzene. The combined benzene extracts were

concentrated to a small volume and chromatographed on a 1.8 x 57.-cm.

column of Florisil. The nitrogen mustard was eluted by benzene in the

first fraction. After the benzene was removed by evaporation, the

product was crystallized from a mixture of ligroin (b.p. 66=75°) and

ether to yield 2.05 g. (49.6 per cent) of yellow crystals, m.p. 107-

108°. A preparation on a smaller scale gave a yield of 53.7 per cent,

m.p. 108-109°.

Anal. Calcd. for $C_{26}H_{24}Cl_2NS$: C, 66.80; H, 5.18; N, 5.99.

Found: C, 66.74, 66.75; H, 5.40, 5.07; N, 5.63, 5.78.

6. 14-{2-[Bis-(2-chloroethyl)amino]ethyl}dibenzo[a,h]phenothiazine

Hydrochloride

Dry hydrogen chloride was passed through a solution of 3.89 g.

(0.00832 mole) of the amine in the minimum quantity of absolute ethanol.

The solution was evaporated to a smaller volume and ether was added until

a permanent cloudiness could be detected. The mixture was placed in the

cold room overnight to allow crystallization of the hydrochloride. The

yield was 3.69 g. (88.2 per cent) of colorless crystals, m.p. 167=175°

at atmospheric pressure and 175-185° in vacuo.

Anal. Calcd. for $C_{26}H_{25}Cl_3N_2S$: C, 61.98; H, 5.01; N, 5.56.

Found: C, 62.00, 61.88; H, 5.02, 4.89; N, 5.41, 5.70.

7. Metalation of 14H-Dibenzo[a,h]phenothiazine

a. 1-Carboxy-14H-dibenzo[a,h]phenothiazine. Ten milliliters

(0.015 mole) of a hexane solution of n-butyllithium was added with

stirring to a suspension of 1.50 g. of 14H-dibenzo[a,h]phenothiazine

in 30 ml. of anhydrous ether under an atmosphere of dry nitrogen.
Initially, a dark red solution was formed from which precipitated a
red crystalline material. The mixture was gently refluxed for 5 hours,
after which it was cooled in a solid carbon dioxide-acetone bath. An
excess of crushed solid carbon dioxide was added and the mixture was
allowed to warm gradually to room temperature. The resulting yellow
suspension was extracted several times with water. The combined
aqueous extracts were washed with ether and then acidified with 50 per
cent hydrochloric acid. The maroon solid which precipitated was removed
by filtration on a fine, sintered glass suction funnel, washed with
water, and dried overnight in a vacuum desiccator. The yield of crude
acid was 1.38 g. (80.2 per cent).

Anal. Calcd. for $C_{21}H_{13}NO_2S$: C, 73.50; H, 3.81; N, 4.07.
Found: C, 73.23, 73.55; H, 4.06, 3.95; N, 4.26, 4.15.

Several determinations were necessary to estimate the melting
characteristics of the compound. A sample was placed in a capillary
tube in the Mel-Temp apparatus which had been heated to a temperature
of 100°. The sample became lighter in color at 150-160°, then turned
black at 255° and melted with decomposition at 265-267°. Samples placed
in the melting-point block at temperatures above 170° melted after a
short induction period and immediately resolidified to a bright red
material. This unusual behavior is explained by the fact that when
the acid is heated, water is eliminated and a new compound, the lactam
of the acid, is formed. Thus, the melting point of 265-267° is not
that of the acid but that of the impure lactam, the formation and

purification of which is described in the following procedure. The acid was found to be moderately soluble in hot, 10 per cent, aqueous sodium carbonate.

b. Lactam of 1-carboxy-14H-dibenzo[a,h]phenothiazine. A procedure similar to the one used in the preparation of this compound was employed by Shirley and Liu[71] in the synthesis of the lactam of 1-carboxy-12H-benzo[a]phenothiazine. In a test tube was placed 0.37 g. (0.00108 mole) of the acid obtained from the metalation of 14H-dibenzo-[a,h]phenothiazine. The test tube containing the acid was heated in a wax bath at a temperature of 190-210° for 8 minutes. Water vapor was observed to condense on the sides of the test tube. The solid resulting from the conversion was recrystallized from benzene to yield 0.24 g. (66.4 per cent) of red-orange needles, m.p. 270-272°.

Anal. Calcd. for $C_{21}H_{12}NOS$: C, 77.55; H, 3.38; N, 4.30. Found: C, 77.32, 77.35; H, 3.46, 3.31; N, 4.22, 4.35.

The lactam was insoluble in both hot and cold 10 per cent aqueous sodium carbonate. Comparison of the infrared spectra of the acid and lactam revealed a shift in the carbonyl stretching band from 6.01 μ for the acid to 5.86 μ for the lactam. Bellamy[75] indicates that such a shift is to be expected.

c. Reaction of 1-carboxy-14H-dibenzo[a,h]phenothiazine with diazomethane. An attempted synthesis of the methyl ester of 1-carboxy-14H-dibenzo[a,h]phenothiazine with diazomethane resulted in the formation of the lactam of the acid. In a mixture of 10 ml. of freshly distilled tetrahydrofuran and 10 ml. of anhydrous ether was dissolved

0.10 g. (0.00029 mole) of 1-carboxy-14H-dibenzo[a,h]phenothiazine. An ethereal solution of diazomethane prepared according to the procedure of DeBoer and Backer[84] was added and the mixture was placed in an evaporating dish under a hood. In about 2 hours orange-red needles began to crystallize from the solution. A few milligrams of the material was removed on the tip of a spatula. The infrared spectrum of the needles proved to be identical with that of the lactam prepared previously by heating the acid at 200°. The evaporating dish was allowed to stand in the hood overnight. The remainder of the red crystalline product, from which most of the solvent had evaporated, was slurried in a few milliliters of ether, removed by filtration, and dried to yield 0.04 g. (42 per cent) of the lactam, m.p. 269-271°. Recrystallization from benzene raised the melting point to 271-273°. A mixture of this compound and the lactam formed by heat treatment, as previously described, melted at 270-272°.

Anal. Calcd. for $C_{21}H_{12}NOS$: C, 77.55; H, 3.38; N, 4.30. Found: C, 76.79, 76.67; H, 3.26, 3.13; N, 4.05, 3.89.

The lactam was obtained in 46 per cent yield in a repetition of the procedure above.

d. Metalation of 12H-benzo[a]phenothiazine. Following the procedure of Shirley and Liu,[71] 4.00 ml. (0.006 mole) of a hexane solution of n-butyllithium was added in 5 minutes to a solution of 0.50 g. (0.002 mole) of 12H-benzo[a]phenothiazine[43] in 15 ml. of anhydrous ether. The bright red solution was stirred (magnetic stirrer) for 100 minutes at room temperature after which it was cooled in a solid carbon dioxide-

acetone bath. The cold solution was carbonated with excess crushed
solid carbon dioxide. The resulting yellow suspension was extracted
several times with water and the combined aqueous extracts were
acidified with 50 per cent hydrochloric acid. The red-brown precipi-
tate was removed by filtration, washed with water and dried to yield
0.35 g. (60 per cent) of the crude acid, m.p. 125-127°. After recrys-
tallization from a mixture of acetone and ligroin (b.p. 100-115°),
the acid melted at 132-133°. Shirley and Liu[71] report a melting point
of 136-137° and a yield of 94 per cent.

e. Reaction of 1-carboxy-12H-benzo[a]phenothiazine with diazo-
methane. A solution of 0.10 g. of the carboxylic acid prepared in the
preceding experiment in a mixture of 10 ml. of tetrahydrofuran and 10
ml. of ether was added to an ethereal solution of diazomethane.[84] The
mixture was placed in an evaporating dish under a hood for several
hours. Ligroin (b.p. 66-75°) was then added and the remaining tetra-
hydrofuran and ether were evaporated on the steam bath. Orange-red
needles, m.p. 114-116°, crystallized from the ligroin solution.

Anal. Calcd. for $C_{18}H_{13}NO_2S$: C, 70.33; H, 4.26; N, 4.55.
Found: C, 70.08, 69.78; H, 4.04, 4.06; N, 4.20, 4.66.

It was evident,from a comparison of the melting point and infrared
spectrum of the product above with the melting point and spectrum of a
sample of the lactam of 1-carboxy-12H-benzo[a]phenothiazine synthesized
by Shirley and Liu,[71] that the reaction proceeded in the normal manner
to produce the methyl ester.

E. Formation and Reactions of 14H-Dibenzo[a,c]phenothiazine

1. 9-Bromophenanthrene

The "Organic Syntheses" preparation of Dornfeld, Callen and Cole-
man[86] was employed in the preparation of 9-bromophenanthrene.

A total of 62.8 g. (57.6 per cent) of pale yellow oil, b.p. 165-
179°/1 mm. was collected. A small amount of the oil was crystallized
from ethanol yielding colorless crystals, m.p. 50-54°. Dornfeld,
Callen and Coleman reported a yield of 90-94 per cent based on material
which distilled at 177-190°/2 mm. The checkers, R. E. Carnahan and H.
Adkins,[86] obtained yields of 80-84 per cent, m.p. 54-56°, which when
recrystallized had a melting point of 65-66° and amounted to a yield
of 60 per cent.

The compound also was obtained commercially from the Aldrich
Chemical Company. This product melted at 55-57°.

2. N-phenyl-9-phenanthrylamine

A procedure similar to the one given below was used by Scardiglia
and Roberts[73] for the preparation of diphenylamine. To 200 ml. of
freshly distilled, refluxing aniline in a 500-ml., three-necked flask
equipped with an air-cooled condenser and a magnetic stirrer was added
7.15 g. (0.183 mole) of potassium metal. During the addition of the
potassium, the evolution of ammonia was detected. The potassium was
added in 50 minutes and the resulting black solution was refluxed for
4 hours. A solution of 45.0 g. (0.175 mole) of 9-bromophenanthrene in
50 ml. of aniline was added in about 1 hour to the refluxing solution

of potassium anilide, after which the mixture was refluxed for 15.5

hours. The mixture was allowed to cool and ethanol was added cautious-

ly. Ether was added and the mixture was extracted several times

with water to remove the potassium bromide. The ether was removed by

evaporation on the steam bath and most of the aniline was removed under

diminished pressure. The black residue was distilled in vacuo and

22.80 g. of pale yellow oil, b.p. 224-228°/0.5 mm., was collected. Two

recrystallizations from ligroin (b.p. 66-75°) yielded 18.97 g. (40.3

per cent) of colorless needles, m.p. 133-134.5°. Wolfram and Schnurr[72]

prepared N-phenyl-9-phenanthrylamine from sodium anilide and 9-chloro-

phenanthrene and reported a melting point of 138°. The melting point

of the compound was not changed from the value of 133-134.5° by chroma-

tographic purification on Florisil or by recrystallization.

Three additional preparations of N-phenyl-9-phenanthrylamine re-

sulted in yields of 37-42 per cent.

3.   14H-Dibenzo[a,c]phenothiazine

A mixture of 10.00 g. (0.0372 mole) of N-phenyl-9-phenanthryl-

amine, 2.40 g. (0.075 g. atom) of sulfur, and 0.10 g. of iodine con-

tained in a 100-ml.,round-bottomed flask was placed in a Wood's metal

bath, the temperature of which was 155°. The first evolution of hydro-

gen sulfide was noted after the melt had reached a temperature of 142°.

The reaction mixture was maintained at a temperature of 180-190° for

20 minutes and at 195-205° for 40 minutes. The warm melt was dissolved

in benzene and the resulting solution was filtered. Ligroin (b.p. 100-

115°) was added until a small amount of dark tar came out of solution.

The resulting amber solution was chromatographed on a 3.0 x 55-cm.
column of Florisil. A large volume of ligroin (b.p. 66-75°) was used
in the elution of the product. A yield of 1.80 g. (16.2 per cent) of
yellow needles, m.p. 160-164°, crystallized from the combined ligroin
eluates. The purest material obtained by several runs of the procedure
above had a melting point of 163-165° with decomposition after two re-
crystallizations from ligroin (b.p. 100-115°).

Anal. Calcd. for $C_{20}H_{13}NS$: C, 80.23; H, 4.38; N, 4.68.
Found: C, 79.83, 79.99; H, 4.33, 4.33; N, 4.69, 4.53.

Difficulty was experienced in the synthesis of 14H-dibenzo[a,c]-
phenothiazine. A number of thionations differing in the time and
temperature of reaction resulted in yields ranging from 4 to 16 per
cent. The conditions and results of these reactions are summarized in
Table IV.. The best yield was obtained from the procedure described
above.

4. 14-Acetyldibenzo[a,c]phenothiazine

To a mixture of 2.0 ml. of acetic anhydride and 0.20 g. of zinc
chloride was added 0.20 g. (0.00067 mole) of 14H-dibenzo[a,c]pheno-
thiazine. The mixture was allowed to react at room temperature for 2
hours, after which it was poured into ice water and allowed to stand
for about 2 hours. The brown solid which precipitated was removed by
filtration, washed with water, and dried on a Büchner funnel. The
material was dissolved in ethanol and the solution was treated with
"Norit-A" decolorizing carbon. A yield of 0.15 g. (65 per cent) of

TABLE IV

EFFECT OF VARIED CONDITIONS ON THE YIELD OF
14H-DIBENZO [a,c] PHENOTHIAZINE

Reaction Temperature	Reaction Time (Min.)	Melting Point of Product	Yield (Per Cent)
120–130°	40	144–147°	9.0
160–170°	40		
160–165°	40	163.5–164.5°	3.9
180°	10		
180–190°	15	135–140°	10.2
195–210°	20		
180–190°	10	155–160°	10.5
195–205°	40		
180–190°	20	160–164°	16.2
195–205°	40		
210–214°	12	152–161°	4.2
180–190°	20	163–165°	11.1
200–205°	40		

colorless product, m.p. 239-241° crystallized from the ethanol. A second recrystallization from ethanol raised the melting point to 242-243.5°.

Anal. Calcd. for $C_{22}H_{15}NOS$: C, 77.39; H, 4.43; N, 4.10. Found: C, 76.99, 77.42; H, 4.18, 4.20; N, 4.53, 4.22.

5. 14-Methyldibenzo[a,c]phenothiazine

To a suspension of 0.50 g. (0.00167 mole) of 14H-dibenzo[a,c]-phenothiazine in 25 ml. of anhydrous ether was added 1.40 ml. (0.0021 mole) of a hexane solution of n-butyllithium. The dark red suspension which formed was stirred at room temperature for 1 hour. A solution of 0.35 g. (0.00188 mole) of methyl p-toluenesulfonate in 10 ml. of anhydrous ether was added in about 3 minutes. The reaction mixture was stirred at room temperature for 14.5 hours, after which the ethereal solution was washed with water to remove the lithium p-toluenesulfonate. Ligroin (b.p. 66-75°) was added and the ether was removed by evaporation. The ligroin solution was chromatographed on a 1.5 x 45-cm. column of Florisil. Ligroin (b.p. 66-75°) eluted the product at a slow rate. A 3:7 mixture of benzene and ligroin (b.p. 100-115°) proved to be a better eluant. A yield of 0.327 g. (62.5 per cent) of yellow 14-methyldibenzo[a,c]phenothiazine, m.p. 118-119°, crystallized from ligroin (b.p. 100-115°) after removal of the benzene.

Anal. Calcd. for $C_{21}H_{15}NS$: C, 80.47; H, 4.83; N, 4.47. Found: C, 80.63, 80.68; H, 4.95, 5.10; N, 4.74, 4.60.

6. 14-Methyldibenzo[a,c]phenothiazine-9-oxide

To a bright yellow solution of 0.110 g. (0.000351 mole) of 14-methyldibenzo[a,c]phenothiazine in 15 ml. of glacial acetic acid was added 1.50 ml. of 30 per cent hydrogen peroxide. A green color developed immediately. Heating on the steam bath caused the color of the solution to change to blue. It then became colorless and finally after 10 minutes of heating, a pale yellow color could be detected. The solution was poured into 100 ml. of water, which caused the precipitation of a tan material. The crude product was collected by filtration and was recrystallized from a mixture of ligroin (b.p. 100-115°) and acetone. A yield of 0.082 g. (70.9 per cent) of colorless crystals, m.p. 204-205°, was obtained. A second recrystallization increased the melting point to 206-207°.

<u>Anal</u>. Calcd. for $C_{21}H_{15}NOS$: C, 76.56; H, 4.60; N, 4.25. Found: C, 76.75, 76.63; H, 4.57, 4.61; N, 4.09, 4.31.

The infrared spectrum of the compound reveals bands at 9.63 and 9.81 microns which are characteristic of the sulfoxide group.

The oxidation of 77 mg. of 14-methyldibenzo[a,c]phenothiazine with 1.5 ml. of hydrogen peroxide (30 per cent) for 1.5 hours under reflux in acetic acid yielded only a few milligrams of a light brown material. Bands in the infrared spectrum at 8.67 and 8.83 microns indicate that the product of the oxidation was the sulfone, 14-methyldibenzo[a,c]phenothiazine-9-dioxide.

7. 14-(2-Chloroethyl)dibenzo[a,c]phenothiazine

In a dry nitrogen atmosphere 1.30 ml. (0.00195 mole) of a hexane solution of n-butyllithium was added to a slurry of 0.421 g. (0.00141 mole) of 14H-dibenzo[a,c]phenothiazine in 25 ml. of anhydrous ether. The addition was accomplished in 5 minutes, after which the resulting dark red suspension was stirred for 1 hour at room temperature. The mixture was cooled in an ice bath and then a solution of 0.46 g. (0.00196 mole) of 2-chloroethyl p-toluenesulfonate in 10 ml. of anhydrous ether was added rapidly. The mixture was stirred for 0.5 hour at 0° and for 16.5 hours at room temperature. The ethereal mixture was washed with water, benzene was added, and the ether was removed by evaporation. Sufficient ligroin (b.p. 100-115°) was added to produce a 2:3 mixture of benzene and ligroin. The resulting solution was chromatographed on a 1.5 x 45-cm. column of Florisil. Ligroin (b.p. 100-115°) eluted the majority of the product and a 3:7 mixture of benzene and ligroin (b.p. 100-115°) eluted the remainder. A yield of 0.264 g. (51.8 per cent) of yellow crystals, m.p. 120-121° was obtained. Three recrystallizations from ligroin raised the melting point to 122.5-123°.

Anal. Calcd. for $C_{22}H_{16}ClNS$: C, 73.02; H, 4.46; N, 3.87. Found: C, 72.75, 72.63; H, 4.23, 4.42; N, 3.80, 3.64.

8. Metalation of 14H-Dibenzo[a,c]phenothiazine

To a suspension of 1.03 g. (0.00344 mole) of 14H-dibenzo[a,c]-phenothiazine, m.p. 163-165°, in 70 ml. of anhydrous ether was added 7.40 ml. of a 1.60 molar hexane solution of n-butyllithium in a period of 5 minutes. A dark red suspension formed initially which, after the

addition of the first 4.5 ml. (0.0072 mole) of the solution of n-butyllithium, became bright orange-red. It was noted that after the mixture had stirred (magnetic stirrer) for 21.5 hours at room temperature the color again became dark red. The addition of 3.0 ml. (0.0048 mole) of n-butyllithium solution produced the orange-red color once more. The solution then was stirred for 0.5 hour after which it was cooled in a solid carbon dioxide-acetone bath and carbonated with excess crushed solid carbon dioxide. The yellow suspension was extracted several times with water. The aqueous extracts were combined, washed with ether and benzene, and acidified with 50 per cent hydrochloric acid. The reddish-brown precipitate was collected and washed with water on a fine sintered glass suction funnel. After drying overnight in a vacuum desiccator, the crude yellow-brown product, m.p. 286-289°, amounted to 0.946 g. (84.5 per cent). Two recrystallizations from benzene yielded gold needles, m.p. 289-290°.

Anal. Calcd. for $C_{21}H_{11}NOS$: C, 77.55; H, 3.38; N, 4.30. Found: C, 77.66, 77.73; H, 3.32, 3.60; N, 4.06, 4.24.

In two prior runs in which the molar ratio of n-butyllithium to 14H-dibenzo[a,c]phenothiazine was 3:1 a very small yield of product was obtained. Therefore the ratio was increased to 5:1 in the reaction described above. It was later found that the nitrogen train used in the earlier metalation was not functioning properly and moisture probably decomposed part of the organometallic.

Comparison of the infrared spectra of the acids and lactams of 12H-benzo[a]- and 14H-dibenzo[a,h]phenothiazine with the spectrum of

the product from the metalation of 14H-dibenzo[a,c]phenothiazine indi-
cated that the lactam rather than the carboxylic acid was isolated.
The elemental analysis confirmed the infrared evidence.

F. Ultraviolet Spectra

The ultraviolet spectra of a number of phenothiazines and related
amines were determined using a Cary Model 14 recording spectrophotometer.
In each case a 50 mg. sample of the compound was dissolved in 100 ml.
of 95 per cent ethanol. This solution was diluted to a concentration of
from 4 to 5 mg./l. of ethanol, depending upon the absorption of the par-
ticular compound. The spectrum in the range 200-400 mμ then was deter-
mined and the values of the molar extinction coefficient were calculated
for each maxima. The spectra are on file in the Department of Chemistry,
The University of Tennessee.

CHAPTER V

SUMMARY

The consideration of the medicinal importance of phenothiazine derivatives and the known cancer chemotherapeutic value of the nitrogen mustards made the synthesis of some nitrogen mustard derivatives of the phenothiazines of particular interest. In the course of this research the N-{2-[bis-(2-chloroethyl)amino]ethyl} derivatives of phenothiazine, 7H-benzo[c]phenothiazine, 7H-dibenzo[c,h]phenothiazine, and 14H-dibenzo-[a,h]phenothiazine were synthesized for anticancer evaluation. Also submitted to the Cancer Chemotherapy National Service Center for testing were the N-(2-chloroethyl) monofunctional nitrogen mustard derivatives of the first three phenothiazines mentioned above. The reactions below illustrate the route followed in the preparation of these compounds. The symbol Z-N-H represents a phenothiazine type nucleus. At present
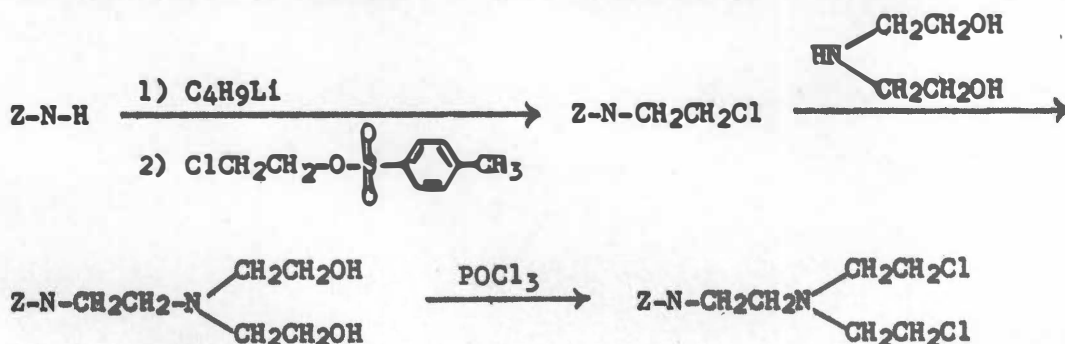
the results of anticancer evaluation of the compounds submitted are incomplete.

The syntheses of 14H-dibenzo[a,c]phenothiazine and some of its N-substituted derivatives were studied.

Additional evidence for the specificity of the metalation of heterocyclic compounds was obtained in a study of the metalation with n-butyllithium of three of the dibenzophenothiazines. In each case the site of metalation was a position adjacent to the nitrogen atom. The three products, 6-carboxy-7H-dibenzo[c,h]phenothiazine, 1-carboxy-14H-dibenzo[a,h]phenothiazine and the lactam of 1-carboxy-14H-dibenzo-[a,c]phenothiazine, were isolated in yields of 77-84 per cent.

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