Negative microfilm 63-6968
received from university

WOOD, Jesse Raymond, 1933-
POTENTIAL ANTICANCER AGENTS.

The University of Michigan, Ph.D., 1963
Chemistry, organic

University Microfilms, Inc., Ann Arbor, Michigan

POTENTIAL ANTICANCER AGENTS

by
Jesse Raymond Wood

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in the
University of Michigan
1962

Doctoral Committee:

Professor Robert C. Elderfield, Chairman
Professor G. Robert Greenberg
Assistant Professor Daniel T. Longone
Professor Charles L. Rulfs
Associate Professor Milton Tamres

To My Parents

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Professor Robert C. Elderfield for suggesting the projects presented here and for his many kindnesses and beneficial suggestions during the course of this research and in the preparation of this treatise. The skill, inspiration, encouragement, and confidence continually afforded by Professor Elderfield will not soon be forgotten.

The financial assistance granted by the U. S. Public Health Service for the years 1959-1962 is gratefully acknowledged. The author is also indebted to the Department of Chemistry of The University of Michigan for the award of a Teaching Fellowship for the years 1955-1959, and for the award of an Albert B. Prescott Fellowship for the summer of 1958. In addition, the award of a University Scholarship by the Horace H. Rackham School of Graduate Studies for the academic year 1955-1956 is greatly appreciated.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	iii
TABLE OF CONTENTS	iv
INTRODUCTION	1
HISTORICAL	2
DISCUSSION	32
Ethylenamides of α-Amino Acids	32
Background	32
Contributions from This Laboratory	33
Indole and Indole-type Nitrogen Mustards	36
Biochemical Aspects	36
Background	36
Organic Chemistry Aspects	39
Problems and Approaches to the Synthesis of Special Nitrogen Mustards	39
Background	41
Contributions from This Laboratory	42
Pyrimidine Nitrogen Mustards	48
Background	48
Contributions from This Laboratory	48
Imidazole and Pteridine Nitrogen Mustards	49
Background	49
Contributions from This Laboratory	51

Page

EXPERIMENTAL 55

Phthaloylglycine 55

Phthaloylglycyl Chloride 55

N-[(1-Aziridinylcarbonyl)methyl]phthalimide. . 55

N-Phenylglycine 56

N-Phenylglycine-N-carboxyanhydride 56

1-(N-Phenylglycine)ethylenamide 56

N-Phenylalanine 57

N-Phenylalanine-N-carboxyanhydride 58

1-(N-Phenylalanine)ethylenamide 58

1-(2'-Chloropropionyl)ethylenamide 59

Bis(2-chloroethyl)amine 59

N-[Bis(2-chloroethyl)aminomethyl]isatin . . . 60

N-[Bis(2-chloroethyl)aminomethyl]phthalimide . 60

9-[Bis(2'-chloroethyl)aminomethyl]carbazole . 61

N-[Bis(2-chloroethyl)aminomethyl]succinimide . 62

N,N-Bis(2'-chloroethyl)-3-indoleglyoxylamide . 62

Purification of p-[N,N-bis(2-chloroethyl)amino]-benzaldehyde 63

3-Indoleacet[4'-bis(2"-chloroethyl)amino]-benzylidenehydrazide 64

3-Indoleglyoxyl[4'-bis(2"-chloroethyl)amino]-benzylidenehydrazide 65

1-(3'-Indoleacet)-2-[4"-bis(β-chloroethyl)amino]-benzylhydrazine 66

β-(3-Indole)propionic Acid 67

β-(3-Indole)propionic Acid Ethyl Ester 67

Page

β-(3-Indole)propiohydrazide 68

β-(3-Indole)propio[4'-bis(2"-chloroethyl)amino]-benzylidenehydrazide 68

Indole-3-carboxylic Acid 69

Indole-3-carboxylic Acid Ethyl Ester 69

Indole-3-carboxylic Acid Methyl Ester 69

Indole-3-carboxhydrazide 70

Indole-3-carbox[4'-bis(2"-chloroethyl)amino]-benzylidenehydrazide 71

N-[4-(Bis((2'-chloroethyl))amino)]benzylidene-tryptamine 71

5-[Bis(2'-chloroethyl)aminomethyl]-6-methyl-uracil Hydrochloride 73

5-[Bis(2'-chloroethyl)aminomethyl]uracil Hydrochloride 74

Imidazole-4,5-di[carbox(4'-bis((2"-chloro-ethyl))amino)benzylidenehydrazide] 74

1-Benzyl-2-[bis(β-hydroxyethyl)aminomethyl]-imidazole 75

dl-4,4'-Diacetamidobenzoin 76

4,4'-Diaminobenzil 77

4,4'-Bis[4"-(bis((β-chloroethyl))amino)-benzylidene]diaminobenzil. 77

BIBLIOGRAPHY 79

INTRODUCTION

Hundreds of alkylating agents (such as ethylenimines and nitrogen mustards) have been synthesized and tested as anticancer agents in recent years. However, certain types of compounds which occupy central positions in biochemistry are conspicuously absent from the list of groups to which alkylating functions have been attached. For example, no pteridine ethylenimines or nitrogen mustards (with one, very recent exception) are known in the literature; this is also the case for imidazoles. Until late in 1961, no indole nitrogen mustards had been reported. Again, no amides of ethylenimine and α-amino acids have been reported, even though the corresponding α-ethylenimino acids are known, as are compounds in which the nitrogen mustard moiety occurs either as an amide function or at the α-amino position.

This work was undertaken in an attempt to prepare ethylenamides of α-amino acids, and also to synthesize certain indole nitrogen mustards, none of which were known when the work was begun. The amino acid work was only partially successful, but excellent results were obtained in the indole project. In addition, two highly desirably pyrimidine nitrogen mustards, one imidazole nitrogen mustard and one imidazole diol, and an intermediate for a pteridine nitrogen mustard have been prepared.

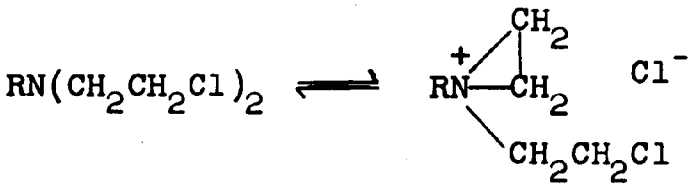
HISTORICAL

Cancer is the name given to a group of over three hundred types of cell disorders, all characterized by abnormal growth. Cancer was recognized as a disease by the Egyptians as early as 1500 B.C.; references to it have been found in early Greek and Sanskrit writings. Through the ages, relief from the disease has been sought through plant and herbal preparations. However, only in recent decades have deliberate attempts and programs been initiated to overcome the disease. Perhaps part of the delay was necessary until the belief arose that it was possible to treat cancer with drugs. The current status of clinical cancer chemotherapy has been reviewed recently (1).

Early observations that mustard gas, bis(2-chloroethyl)-sulfide, caused a lowering of the white blood cell count suggested that such compounds might be of value in treating leukemias, since these diseases exhibit excessively high white blood cell counts. The sulfur mustards proved to be highly toxic, and attention was logically turned to the corresponding nitrogen mustards. Early clinical work was done under wartime secrecy, and was first revealed by Gilman and Philips in 1946 (2). Since then, the nitrogen mustard group has been attached to many types of compounds, including amino acids, carbohydrates, steroids, and many heterocyclic systems. The biological activity of these mustards varies considerably. Some are quite active and

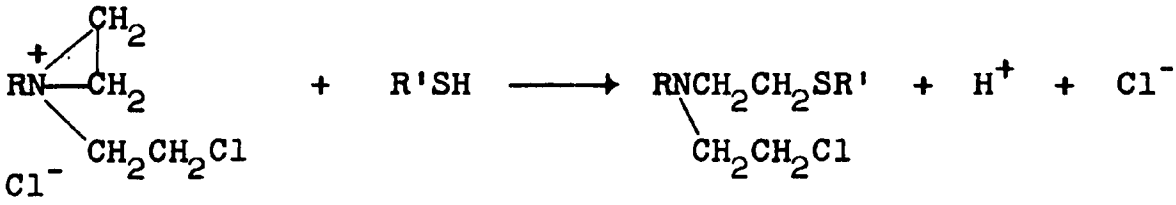
useful, but are often limited by the development of drug resistance and by toxicity to other tissues, especially those tissues which proliferate rapidly (3,4). Some of the biological effects of nitrogen mustards are also produced by x-rays (3).

The nitrogen mustard group is especially well-suited for structural modifications, since the rate-controlling step in its alkylation of a substrate is the formation of a cyclic ethylenimmonium ion (I), whose rate of formation normally depends on the basicity of the nitrogen atom. Thus, a nitrogen mustard group connected to an aliphatic chain is much more reactive than one connected to an aromatic ring. Further, the basicity of an aromatic amino group can be carefully controlled by appropriate substitution in the aromatic ring.



I

The ethylenimmonium ion (I) is the species which alkylates the substrate. The substrate may be an alcohol, mercaptan, amine, carboxylate ion, phosphoric acid residue, etc.



In a whole animal, many molecular sites are attacked by an alkylating agent. Numerous studies have been made to determine the specific reaction(s) responsible for the anticancer action of certain alkylating agents, but no conclusive answer has been obtained so far. Some of the data which led to postulated mechanisms of action have been reviewed (3). A recent study (5) indicates that attack on deoxynucleic acid itself (such as esterification of phosphoryl hydroxyl groups, cross-linking, or removal of guanine) does not necessarily represent the mechanism of action by which alkylating agents exert their anticancer effects. In fact, the results of that study were thought to support the hypothesis that the mechanism of action is based on interference with protein synthesis rather than on the direct inactivation of deoxynucleic acid.

The anticancer action of a drug presumably must arise from some difference(s) between normal and cancerous cells (unless, for example, the causal agent in a cancer is a Mendelian gene mutation or a virus. Even then, drugs may be useful. A few virus diseases can be treated or cured with chemicals). Unfortunately, no new abilities of cancerous cells have been discovered so far, with one

exception. There are reports (6) that in contrast to their normal counterparts, acute leukemic cells (of mice and children) are able to synthesize folic acid and the anti-cancer drug "aminopterin", when supplied with the appropriate precursors. There is also an indication (7) that some mouse tumors, but not the host animal, can synthesize vitamin B_{12}.

Most of the differences between normal and cancerous cells are of a quantitative nature.

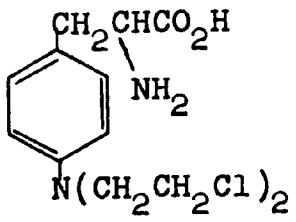
1. The ability of tumors to maintain aerobic as well as anaerobic glycolysis at sustained high levels does not seem especially amenable to attack by drugs (8). Also, some tumors do not exhibit this phenomenon, and some normal tissues do show it (8). (However, the lactic dehydrogenase approach (9) seems quite valid.)
2. Many anticancer drugs have been designed to inhibit reactions (especially nucleic acid synthesis) which are essential for cell reproduction. Such an approach may have serious disadvantages, since the differential action of such a drug must be based on the faster rate of growth of a tumor system (although some normal cells also proliferate rapidly) or on a greater susceptibility of a tumor system to the drug (8). Danielli (10) assumes that the difference between normal and tumor cells with respect to any one variable is insufficient

to obtain an adequate therapeutic index, if all tumor cells are to be killed without intolerable damage to the organism as a whole. He has therefore designed drugs whose action might depend on two or more variables.

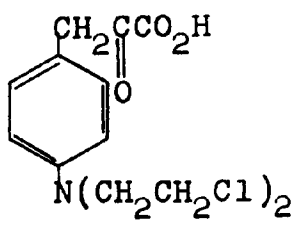
3. A fairly promising but almost untried approach is the design of drugs to exploit various enzymatic deficiencies of cancerous cells, or stated another way, to exploit the completeness of normal cells. This approach is based on the observation that tumors of the most diverse origins tend to converge to a common cell type; that is, in their biochemical characteristics, tumors resemble one another more closely than they resemble their tissues of origin (11). Also, an experimentally observed corollary of this so-called "Greenstein generalization" is that some enzymes, particularly those involved in specific functional activities of normal tissue, are nearly or completely lost in neoplasms (11). It should be kept in mind, however, that merely a difference between a tumor and its normal homologue is not ideal for chemotherapy; there should be differences between the tumor and <u>all</u> normal tissues of the body (12). Some such situations are known (for example, the enzyme transaminase) (13). On the other hand, considerably enhanced drug action might result even if only a few certain normal tissues

(for example, the rapidly proliferating ones) could detoxify a given drug.

Some anticancer drugs may owe their action to higher levels of certain enzymes in cancerous tissue. (Prostatic carcinomas have a high acid phosphatase activity, and the estrogenically inert stilbestrol diphosphate is used in their palliative treatment; stilbestrol itself shows a marked cytostatic action (14). The effective drug cyclophosphoramide (15,16) is a similar case.) Another promising approach is the design of a toxic drug which a) can be detoxified by some enzyme(s) present in normal cells, but which is lethal to the cancerous cells because they lack the enzyme(s) necessary to metabolize it to a non-toxic substance; or which b) is administered in a dose such that some important tumor enzyme (present in decreased amount) is inhibited, while the same enzyme in normal cells is only partially affected because much more of it is present (13). A test of this approach was recently made (17). The working hypothesis was that the "phenylalanine mustard" (II) is effective because cancerous cells lack the phenylalanine transaminase to convert it to the non-toxic phenylpyruvate derivative (III).



II



III

(One group had shown that phenylalanine transaminase was essentially absent in nine out of fifteen rodent tumors, but was present in all normal rodent tissues that were examined.) If this hypothesis is true, then III itself should be inactive. The compound was synthesized, but unfortunately no biological results were recorded in that publication.

Two more examples have recently been cited (8) in which low levels of certain tumor enzymes may be responsible for drug action. One of the two places where Amethopterin and aminopterin interfere with normal folic acid action is the enzyme folic acid reductase; acute leukemic cells of the mouse barely have adequate amounts of this enzyme. Again, 6-azauridine has no apparent deleterious effect on normal human cells, but has a lethal effect (at least for awhile) on a few types of human leukemic cells. It seems likely that there is little dependence on <u>de novo</u> synthesis of pyrimidines in normal human cells, but a biochemical defect in the utilization of preformed pyrimidines in leukemic cells forces dependence on <u>de novo</u> synthesis, which is blocked by 6-azauridine-5'-phosphate (by competitive inhibition of orotidylic acid decarboxylase).

4. Many other approaches to anticancer drug design are being studied (methods of drug administration, combination

therapy, etc.) which cannot be discussed here. A few which might be mentioned are the design of drugs to take advantage of the higher acidity of tumor cells (18); attempts to direct a drug to a specific location in the body (19); induction of tumor enzymes followed by treatment with otherwise inefficient drugs (10); use of other variables such as permease and active transport processes, and pinocytosis (10); use of the significantly higher negative charge density of cancerous cells (20) (which, interestingly, rises with increasing malignancy); and use of other differences of cancerous cells (11,21,22).

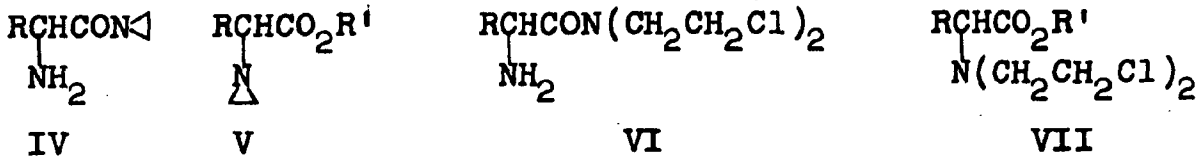
Many synthetic routes have been used to obtain molecules containing the nitrogen mustard group. Some routes of proven or potential generality are illustrated below.

		Ref.
A.	$RX \rightarrow RN(CH_2CH_2OH)_2 \rightarrow RN(CH_2CH_2Cl)_2$	23,24
B.	$-NH_2 \rightarrow -N(CH_2CH_2OH)_2 \rightarrow -N(CH_2CH_2Cl)_2$	24-26
C.	$-COOH \rightarrow -CON(CH_2CH_2Cl)_2 \rightarrow -CH_2N(CH_2CH_2Cl)_2$	27
D.	$-COOH \rightarrow -CONHNH_2 \rightarrow -CONHN{=}CH\text{-}C_6H_4\text{-}N(CH_2CH_2Cl)_2$	19
E.	$-NH_2 \rightarrow -N{=}CH\text{-}C_6H_4\text{-}N(CH_2CH_2Cl)_2$	28
F.	$-NHNH_2 \rightarrow -NHN{=}CH\text{-}C_6H_4\text{-}N(CH_2CH_2Cl)_2$	29
G.	$>CH_2 \rightarrow >C{=}CH\text{-}C_6H_4\text{-}N(CH_2CH_2Cl)_2$	30
H.	$>CH_2 \rightarrow >C{=}N\text{-}C_6H_4\text{-}N(CH_2CH_2Cl)_2$	31,32
I.	$>C{=}O \rightarrow >C{=}N\text{-}C_6H_4\text{-}N(CH_2CH_2Cl)_2$	28
J.	$RCOOH \rightarrow RCONH\text{-}C_6H_4\text{-}N(CH_2CH_2Cl)_2$	33

		Ref.
K.	$\rangle C{=}O \rightarrow \rangle C{=}N\text{-}N(CH_2CH_2Cl)_2$	34,35
L.	Mannich reactions with $HN(CH_2CH_2OH)_2$	36,37
	Mannich reactions with $HN(CH_2CH_2Cl)_2$	38-42
M.	$RNCO \rightarrow RNHCON(CH_2CH_2Cl)_2$	22,43
N.	$ROCOCl \rightarrow ROCON(CH_2CH_2Cl)_2$	44
O.	$RN_2^+ \rightarrow RN{=}N\text{-}N(CH_2CH_2Cl)_2$	45,46
P.	$-C{\equiv}C- \rightarrow -CH{=}\overset{|}{C}N(CH_2CH_2Cl)_2$	47
Q.	The Strecker amino acid synthesis with bis-(2-chloroethyl)amine was unsuccessful in three out of four attempts.	48

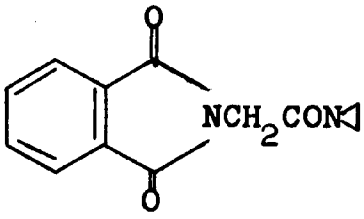
Since the first step in the reaction of a nitrogen mustard with a substrate is formation of a cyclic ethylenimmonium ion (I), interest naturally arose in ethylenimine compounds themselves as possible anticancer agents. Several useful drugs of this type have been developed. It is interesting that some (but not all) of them contain two or more ethylenimine functions.

Partly because of the high activity of the "phenylalanine mustard" (II), various other ways of introducing alkylating functions into amino acid structures were sought. There are four combinations of the ethylenimino or nitrogen mustard function introduced at either the amido or α-amino position.

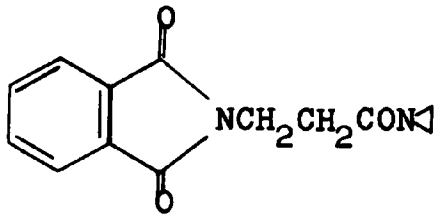


IV V VI VII

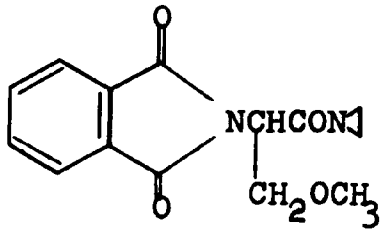
No true examples of type IV have been described or reported in the literature. Fles and Markovac-Prpic (49) prepared the ethylenamides of glycine (VIII), β-alanine (IX), and O-methylserine (X), with the protective phthalolyl group attached in each case, from the corresponding acid chloride. These intermediates were used for further synthesis, and were not tested for biological activity.



VIII

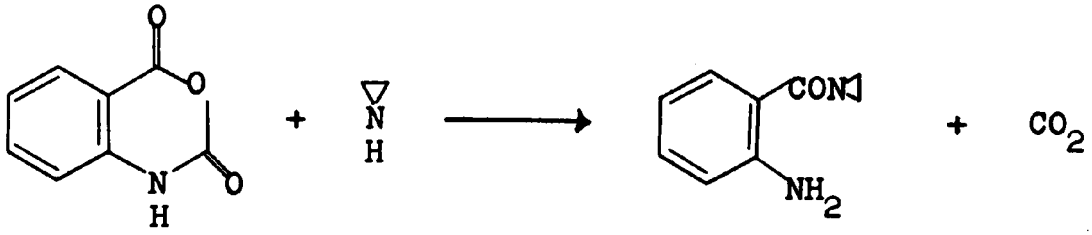


IX

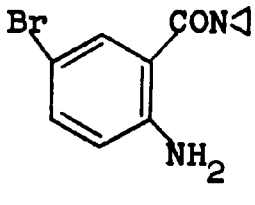


X

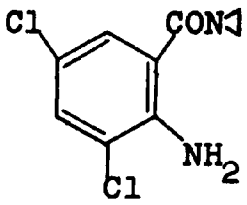
Peterson and Gauss (50) patented several aminoethylenamides (XII-XVIII), most of which were prepared from the appropriate isatoic anhydride (XI) and ethylenimine. (XVIII was obtained from N-carboxy-N-phenylglycine anhydride, but no analyses were reported.) The compounds were intended as intermediates, and no biological properties were given.



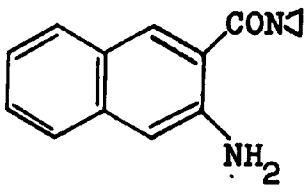
XI XII



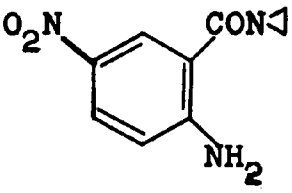
XIII



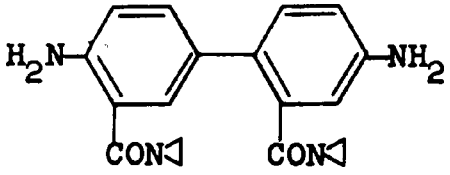
XIV



XV



XVI

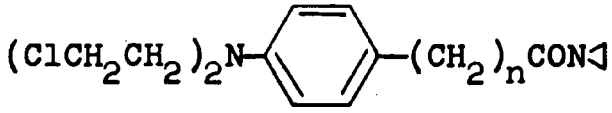


XVII



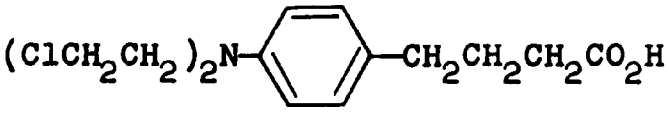
XVIII

Also of interest are compounds of the type XIX (51) based on the anticancer drug "Chlorambucil" (XX), and prepared directly from the appropriate acid and ethylenimine, using dicyclohexylcarbodiimide (no screening results mentioned in the abstract).



XIX

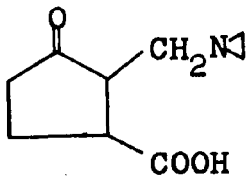
n = 0,1,2, or 3



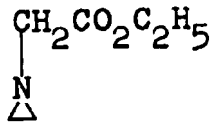
XX

A few examples of type V compounds are known. It is interesting that an ethylenimino group can occur in the same molecule with a carboxyl group, even though ethylenimines are

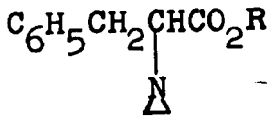
very sensitive to acid. Tsuchiki, Wada, and Suzuki (41) obtained XXI by a Mannich reaction; one of their purposes was to prepare anticancer agents, but no screening results were reported in their patent. Plotz and Weinhardt (52) prepared XXII (not as an anticancer agent) from ethyl chloroacetate. Sunagawa, Murayama, and Yoshida (53) similarly obtained XXII-XXV (in addition to a few other types). They reported that these compounds failed to show a clear effect in the treatment of abdomen tumors in mice. Bestian, Ehlers, Plotz, and Steinbrunn (54) prepared XXVI from methyl acrylate, and presumably XXVII from both maleic and fumaric ester (no indication of biological tests). Vierling (55) reacted certain carbonyl compounds with ethylenimine; ethyl acetoacetate reacted to give ethyl bis(ethylenimino)butyrate (no biological tests indicated). Bestian (56) obtained XXVIII-XXXIII, starting in each case from the corresponding unsaturated compound (no indication of biological tests).



XXI

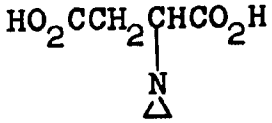


XXII

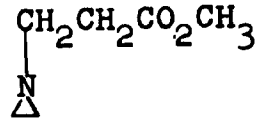


XXIII R = H

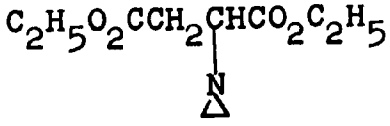
XXIV R = C_2H_5



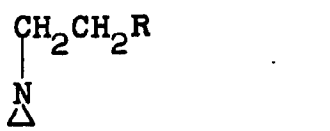
XXV



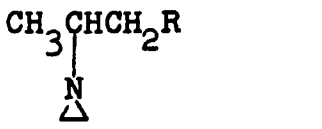
XXVI



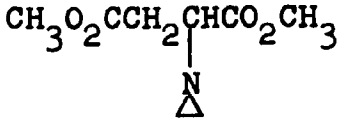
XXVII



XXVIII R = CO_2CH_3
XXIX R = CN
XXX R = $CONH_2$

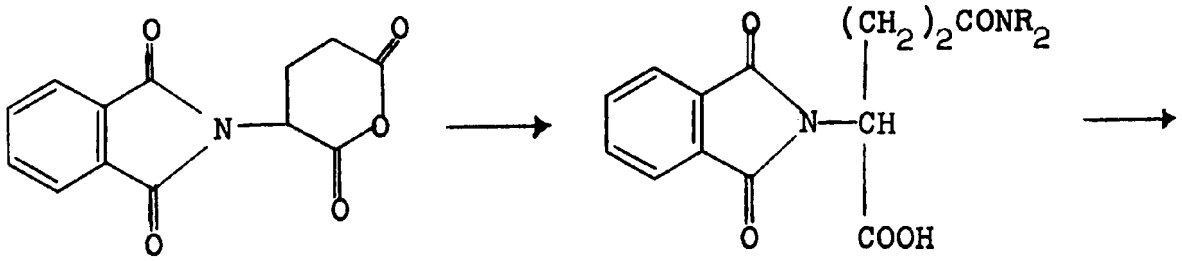


XXXI R = CO_2CH_3
XXXII R = $CONH_2$



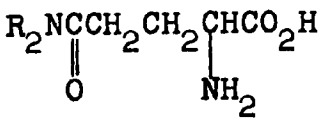
XXXIII

Only one example of a type VI compound is known. Perhaps there are considerable difficulties in preparing such compounds. Friedman and Chatterji (16) reacted various amines with phthaloyl glutamic anhydride (XXXIV) as a prelude to obtaining nitrogen mustards corresponding to XXXVI. However, the nitrogen mustards have not been reported yet.



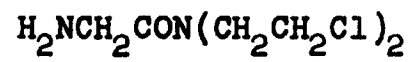
XXXIV

XXXV



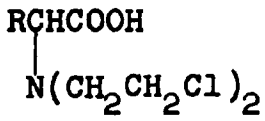
XXXVI

Safonova and Sergievskaya (57) claimed five N,N-di(hydroxyethyl) amides of protected amino acids, but gave physical constants for only two of them, and for only one nitrogen mustard (XXXVII). No screening results were given.



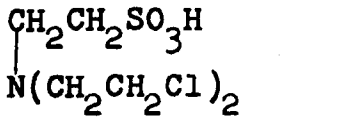
XXXVII

Several examples of type VII compounds are known. Japanese workers (48, 58-60) have prepared them by various routes and have found that they show fairly interesting anticancer activity. Various corresponding nitriles, amides, esters, and N-oxides were also prepared.

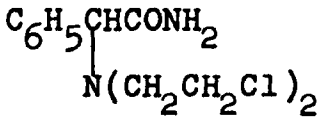


XXXVIII R = H

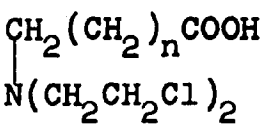
XXXIX R = CH_3



XL

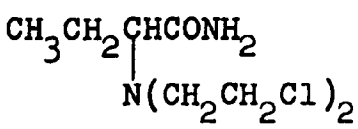


XLI

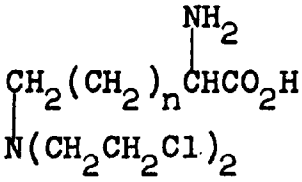


XLII n = 1

XLIII n = 2



XLIV

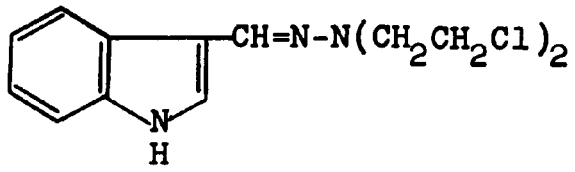


XLV n = 2

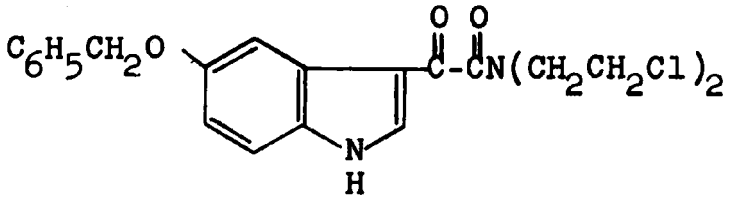
XLVI n = 3

Nyland and Busch (42) reported some compounds of this type in a news release, but did not publish their work; they claimed the compounds had good anticancer activity.

Fourteen indole nitrogen mustards appear in the literature at this time. Schulze and Letsch (35) obtained XLVII from indole-3-aldehyde and 1,1-bis(2'-chloroethyl)-hydrazine (no biological results were given). Kondo, Kataoka, and Dodo (61) prepared XLVIII from the corresponding acid chloride.

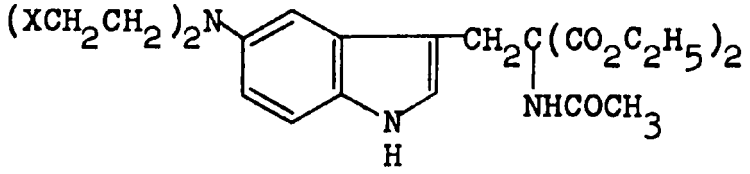


XLVII



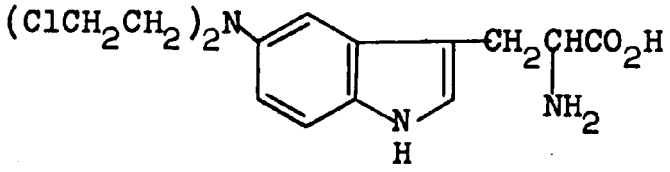
XLVIII

DeGraw and Goodman (62) treated XLIX with thionyl chloride, and as expected, received only dark, intractable products. Reaction of XLIX with methane sulfonyl chloride in pyridine (in order to obtain the corresponding methane sulfonic acid ester) surprisingly produced L, which was then hydrolyzed to LI. Later (63), they used the same approach to prepare LII-LV. No screening reports were given for any of these products.

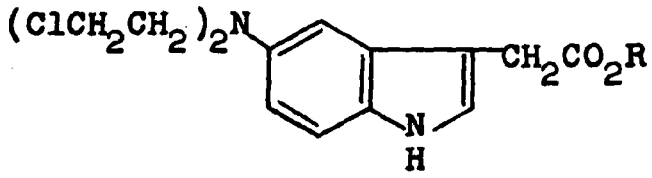


XLIX X = OH

L X = Cl

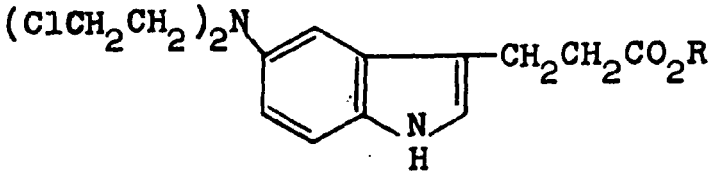


LI



LII R = CH_2CH_3

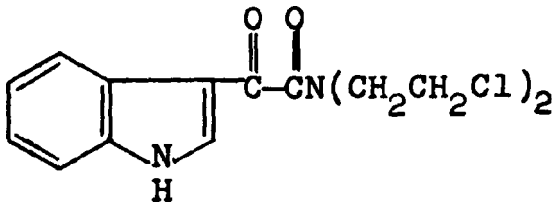
LIII R = H



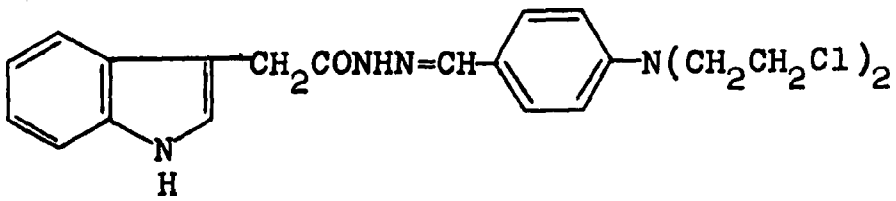
LIV R = CH_2CH_3

LV R = H

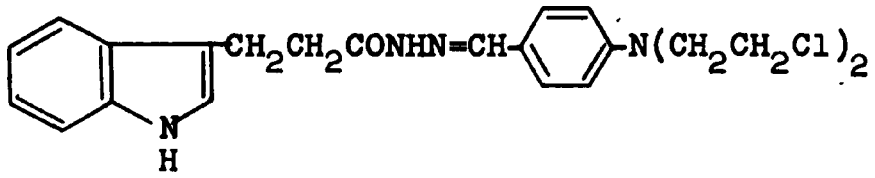
As described later in this treatise, Elderfield and Wood (64) prepared LVI-LXI. In addition, this treatise describes one indole nitrogen mustard, LXII, which does not appear in the literature.



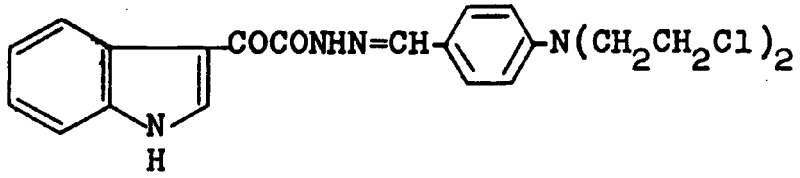
LVI



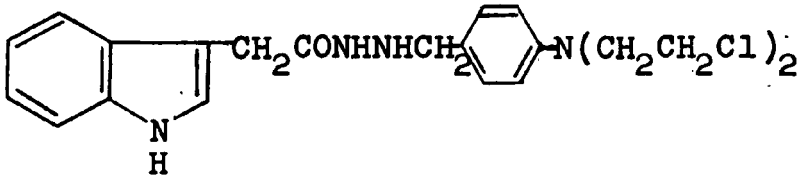
LVII



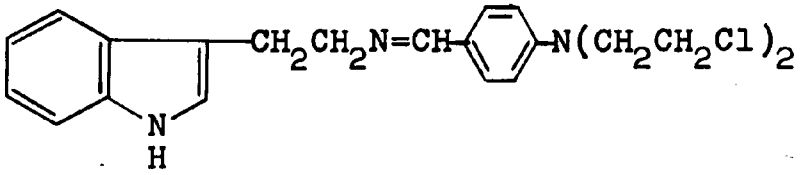
LVIII



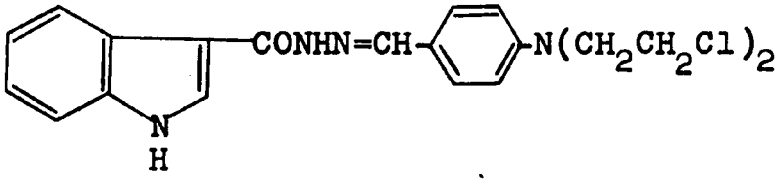
LIX



LX



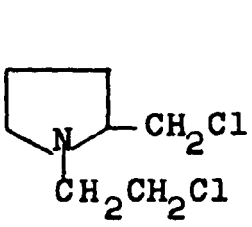
LXI



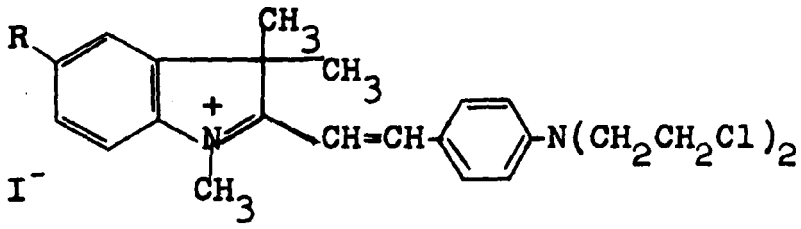
LXII

Ten nitrogen mustards "structurally related" to the indole system are known, discounting LXIII (reported to have a high toxicity) (65), and discounting isoxazole types. Nagaraja and Sunthanker (66) recently prepared LXIV and LXV as dyes. LXVI is also known (67); of course it is incapable

of forming the ethylenimmonium ion (I).

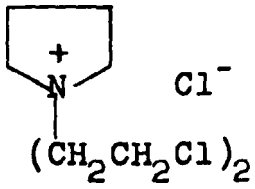


LXIII



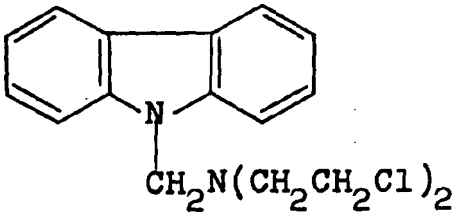
LXIV R = H

LXV R = Cl

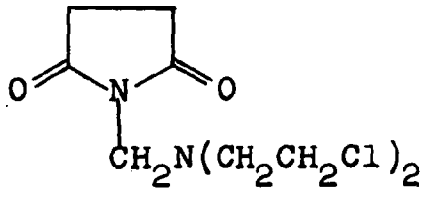


LXVI

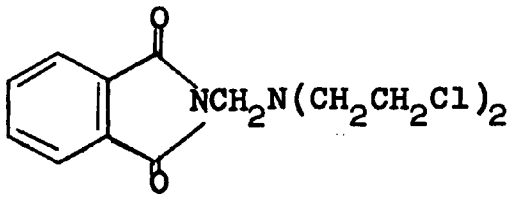
As described later in this treatise, Elderfield and Wood (64) prepared LXVII-LXX. Pettit and Settepani (68) also reported LXIX and LXX, along with LXXI and LXXII. All six of these compounds resulted from Mannich reactions with bis(β-chloroethyl)amine (no screening results given). Schulze and Letsch (69) obtained LXXIII by the action of phthaloyl chloride on 1,1-bis(2'-chloroethyl)hydrazine (no screening results). Preussmann (163) obtained it by chlorination of the diol.



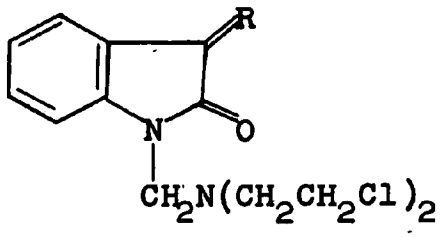
LXVII



LXVIII

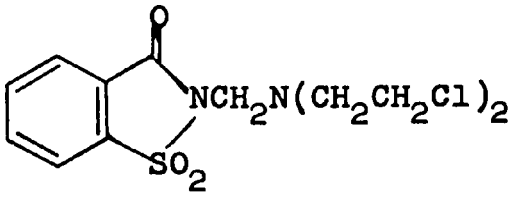


LXIX

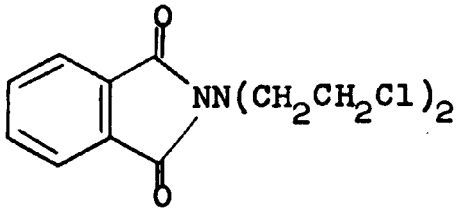


LXX R = O

LXXI R = $NNHCSNH_2$

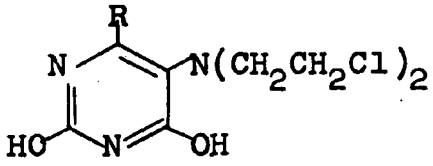


LXXII



LXXIII

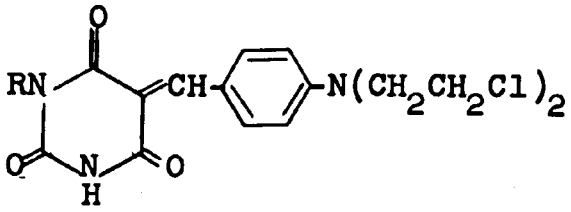
Twenty-seven pyrimidine nitrogen mustards are known in the literature. (Several ethyleniminopyrimidines are known. Some have been used for many years in the treatment of paper, cloth, etc. Certain monochloroethylaminopyrimidines are also known.) Both "Dopan" (LXXIV) (70,71) and the "Upjohn Uracil Mustard" (LXXV) (72) have received widespread testing and study, and interest in them has led to further work in the pyrimidine series. Both were prepared by reacting the primary amine with ethylene oxide, followed by chlorination.



LXXIV R = CH_3

LXXV R = H

LXXVI was prepared by Elderfield, Covey, Geiduschek, Meyer, Ross, and Ross (19) directly from barbituric acid and p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (no screening results were given). LXXVI and also LXXVII-LXXXIII were prepared similarly by Chou, Lin and Yuan (73), who tabulated the carcinostatic action of these compounds.

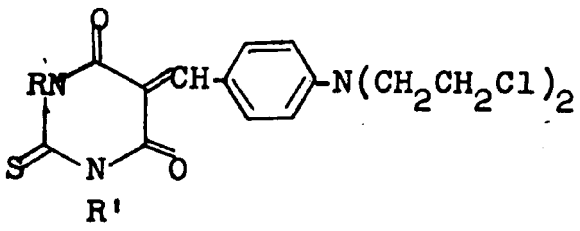


LXXVI R = H

LXXVII R = CH_3

LXXVIII R = C_2H_5

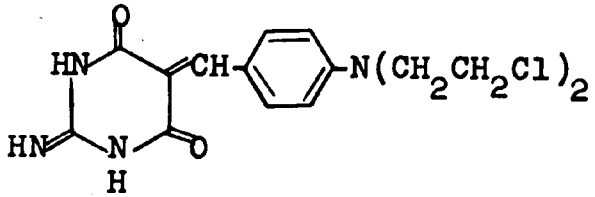
LXXIX R = C_6H_5



LXXX R = R' = H

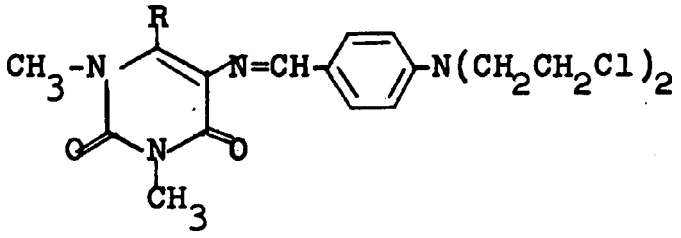
LXXXI R = H, R' = CH_3 (?)

LXXXII R = R' = C_6H_5



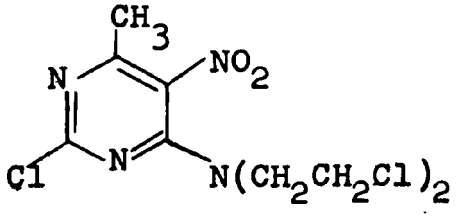
LXXXIII

LXXXIV-LXXXV were reported in a survey of nitrogen mustards (74).



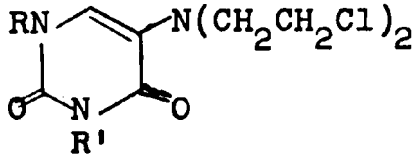
LXXXIV R = H

LXXXV R = CH_3



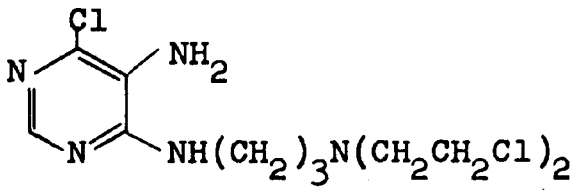
LXXXVI

Benitez, Ross, Goodman, and Baker (75) obtained LXXXVII and LXXXVIII by different routes, each involving chlorination of the diol as the final step (no screening results given). Price and Lin (76) reported LXXXIX (apparently inactive).



LXXXVII R = H, R' = CH_3

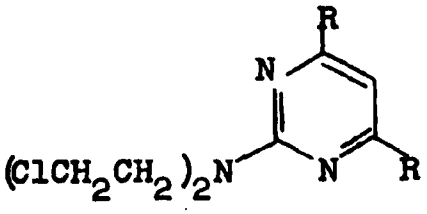
LXXXVIII R = CH_3, R' = H



LXXXIX

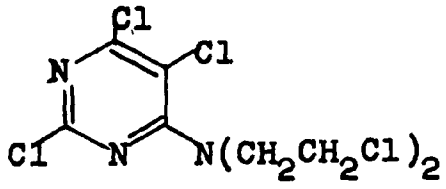
Kornev and Khomenkova (77) prepared four pyrimidine nitrogen mustards (XC-XCIII); XC and XCI could be obtained directly from bis(2-chloroethyl)amine and the corresponding 2-chloro-

pyrimidine.

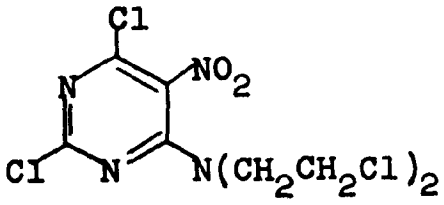


XC R = Cl

XCI R = OH

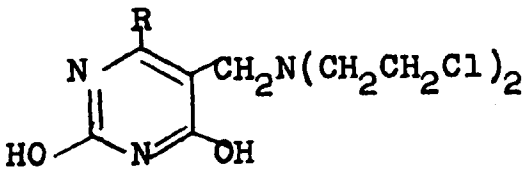


XCII



XCIII

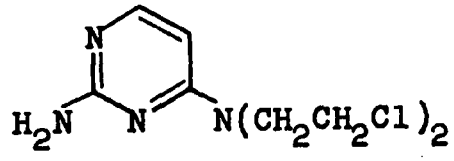
As described later in this treatise, Elderfield and Wood (38) prepared XCIV and XCV by Mannich reactions with bis-(2-chloroethyl)amine (no screening results given); Farkas and Sorm (78) obtained XCIV from bis(2-chloroethyl)amine and 5-chloromethyluracil (and reported it had marked cytostatic activity); and Ross, Lee, Schelstraete, Goodman, and Baker (79) obtained XCIV by chlorination of the diol. Later, Sorm and Vesely (80) showed that the anticancer effects of 6-azauridine and 6-azacytidine are increased by XCIV.



XCIV R = H

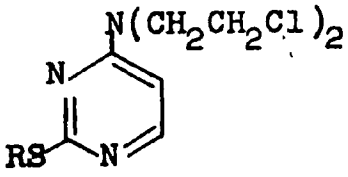
XCV R = CH_3

Lyttle and Petering (81) stated that XCVI produced some effects in selected micro-organisms.



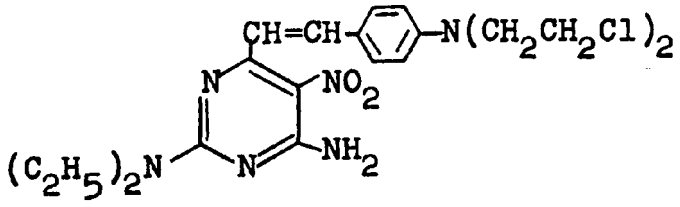
XCVI

Segal and Skinner (82) reported that the two thiocytosine nitrogen mustards XCVII and XCVIII exhibited only slight anticancer properties. (Chlorination of the diols produced the final products.) Lister and Timmis (83) prepared XCIX, but did not carry this particular derivative on to the pteridine and purine (no biological results mentioned for this compound). C has also been reported (84).

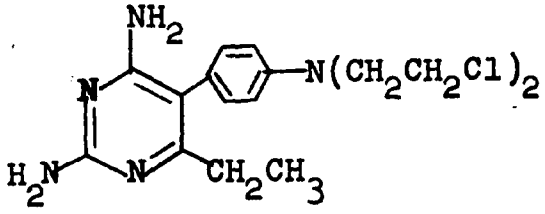


XCVII R = H

XCVIII R = HO_2CCH_2

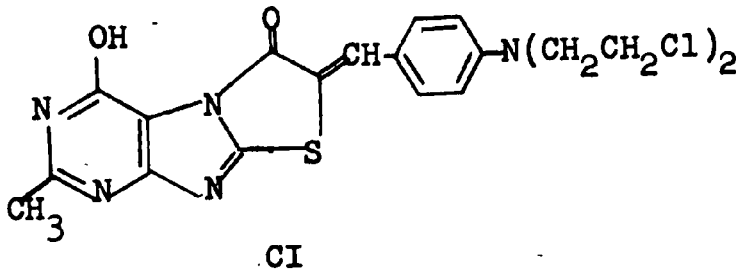


XCIX

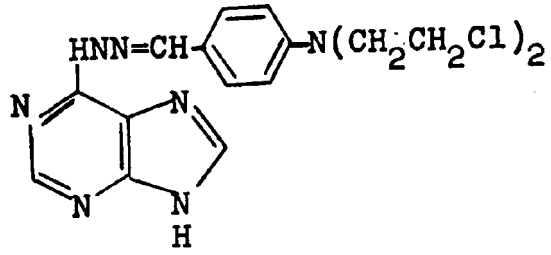


C

Twenty-seven purine nitrogen mustards have been described in the literature. (A few ethyleniminopurines and purine sulfur mustards are also known.) Elderfield and Prasad (85) prepared CI (inactive against the Dunning leukemia) (86). CII was listed in a survey of nitrogen mustards (74).

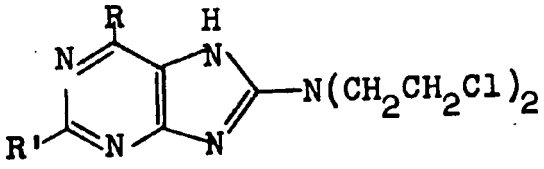


CI



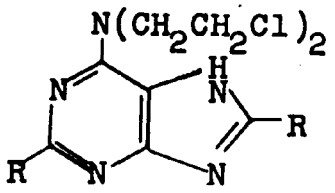
CII

Chu, Harris, and Mautner (87) obtained CIII-CV (no screening results given) by reacting diethanolamine with the 8-halopurine, followed by chlorination.



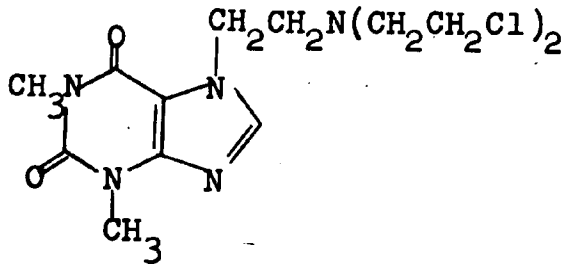
	R	R'
CIII	OH	OH
CIV	OH	H
CV	NH_2	H

DiPaco and Tauro (88) reported CVI-CVIII; there are indications that CVI (prepared by chlorination of the diol, as were the other two) does not have the structure assigned to it (89,90). More recent work (91,92) confirms an alternate structure. CVIII was also obtained by the action of tri-(2-chloroethyl)amine on the potassium salt of theophylline (88). Lyttle and Petering (90) claimed in their patent that CVI possesses mutagenic properties, but Burstein and Ringold (91) reported that it had not demonstrated any significant antitumor activity.



CVI R = H

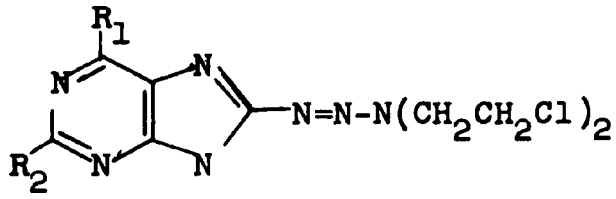
CVII R = Cl



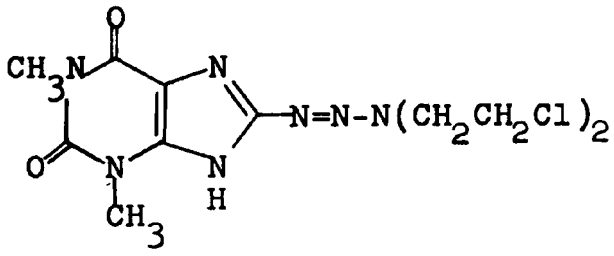
CVIII

Usbeck, Jones, and Robins (45) reacted various 8-diazopurines with diethanolamine or bis(2-chloroethyl)amine, and eventually received CIX-CXIII. CXIV resulted from coupling 8-diazoxanthine with N-phenyldiethanolamine, followed by chlorination. Several of the 8-triazenopurine

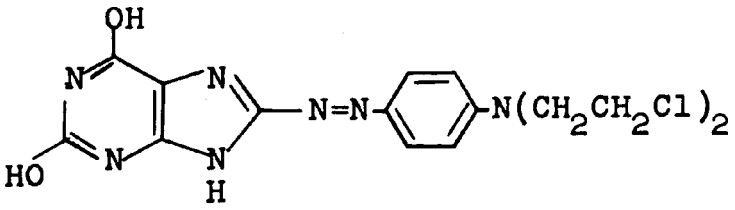
nitrogen mustards showed significant antitumor activity.



	R_1	R_2
CIX	NH_2	H
CX	OH	H
CXI	OH	OH
CXII	OH	NH_2



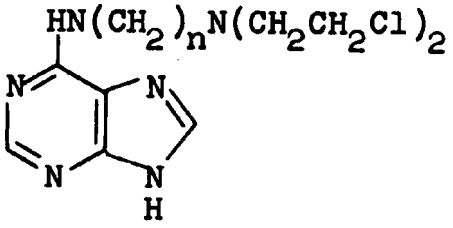
CXIII



CXIV

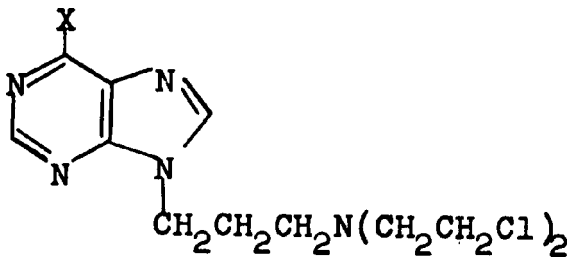
Lin and Price (93) reported that CXV and CXVI have moderate mustard-like activity against a number of mouse tumors. They also prepared (76) CXVII and CXVIII (again from the diols), and found that CXVII demonstrated an interesting spectrum of activity against a number of experi-

mental mouse tumors.



CXV n = 2

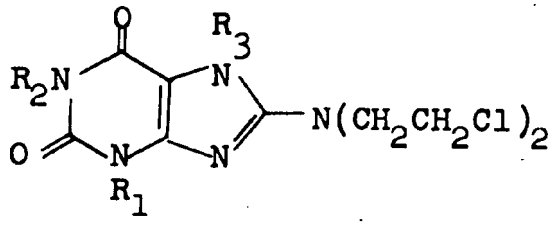
CXVI n = 3



CXVII X = OH

CXVIII X = NH_2

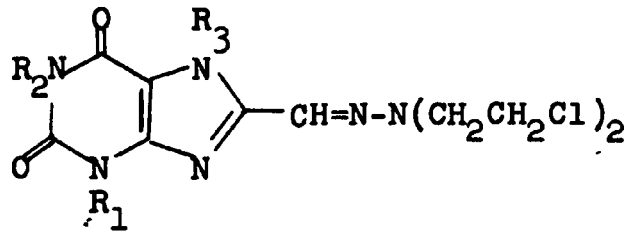
Indications of the activity of CXIII against a spontaneous mouse tumor led Koppel, Springer, Robins, Schneider, and Cheng (94) to prepare CXIX-CXXVI (no screening results given). CXXVII was reported by Glushkev, Golovchinskoya, and Magidson (95) (no screening results given). All nine of these compounds were prepared from the corresponding diols.



CXIX $R_1 = R_2 = CH_3$, $R_3 = H$

CXX $R_1 = R_3 = CH_3$, $R_2 = H$

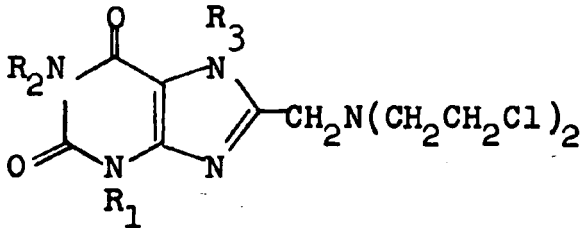
CXXI $R_1 = R_2 = R_3 = CH_3$



CXXII $R_1 = R_2 = CH_3$, $R_3 = H$

CXXIII $R_1 = R_3 = CH_3$, $R_2 = H$

CXXIV $R_1 = R_2 = R_3 = CH_3$

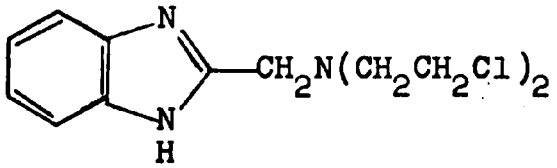


CXXV $R_1 = R_2 = CH_3$, $R_3 = H$

CXXVI $R_1 = R_3 = CH_3$, $R_2 = H$

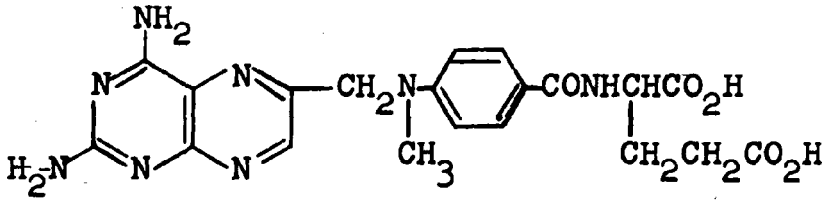
CXXVII $R_1 = R_2 = R_3 = CH_3$

No imidazole nitrogen mustards appear in the literature at the present time, even though in past years considerable interest was displayed (74,96-98) in benzimidazole nitrogen mustards, especially of the general type CXXVIII.

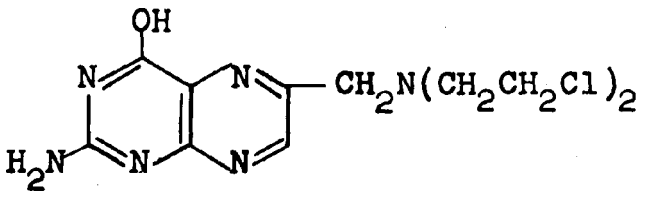


CXXVIII

Only one pteridine nitrogen mustard is known at this time, even though pteridine antimetabolites such as Amethopterin, CXXIX, have been widely used in the treatment, and more recently in the cure, of certain types of cancer. Pushkareva and Alekseeva (99) prepared CXXX by chlorination of the corresponding diol, and also by alkylation of bis-(2-chloroethyl)amine with 2-amino-4-hydroxy-6-bromomethyl-pteridine.



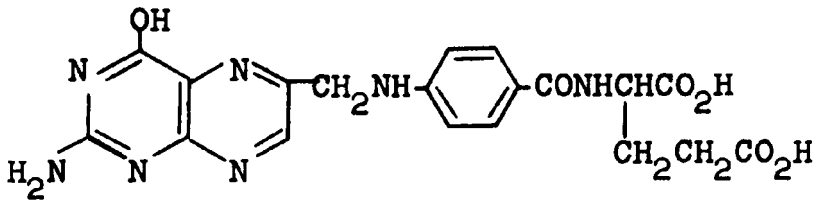
CXXIX



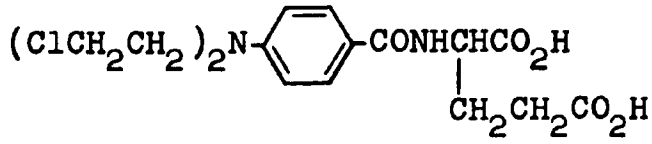
CXXX

Two types of nitrogen mustards have been prepared which are based on p-aminobenzoylglutamic acid, the side chain of the naturally occurring folic acid (CXXXI). Alekseeva and Pushkareva (100) and Fu (101) prepared the dipeptide CXXXII, along with various esters. Fu (101) reported that both the L and D isomers of CXXXII, along with their diethyl esters, showed moderate inhibitory activity on five microbiological systems. Alekseeva and

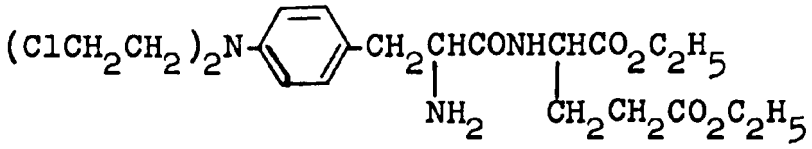
Pushkareva (102) prepared a dipeptide (CXXXIII) (along with its N-formyl and N-acetyl derivatives) based on diethyl glutamate and the known anticancer drug, "phenylalanine mustard" (II) (no screening results given).



CXXXI



CXXXII



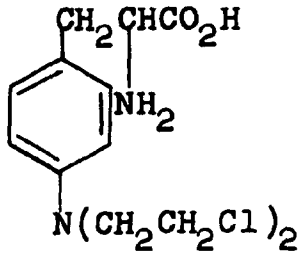
CXXXIII

DISCUSSION

Ethylenamides of α-Amino Acids

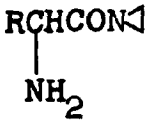
Background

Partly because of the high anticancer activity of the "phenylalanine type of mustard" (II), various other ways of introducing alkylating functions into amino acid residues have been sought.



II

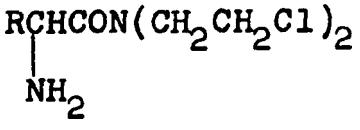
There are four possible combinations of an ethylenimino or nitrogen mustard function introduced at the amido or α-amino position (IV-VII).



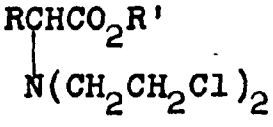
IV



V



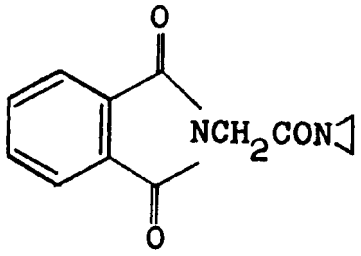
VI



VII

Examples of types V, VI, and VII have been described in the literature. No true examples of type IV are known, although certain closely related compounds have been prepared by various workers.

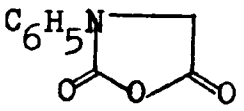
Fles and Markovac-Prpic (49) prepared VIII and other similar compounds from the corresponding acid chlorides and ethylenimine. These products were used as intermediates. Peterson and Gauss (50) claimed in a patent that the α-phenylaminoethylenamide XVIII resulted when the N-carboxyanhydride CXXXIV (prepared from N-phenylglycine and phosgene) was reacted with ethylenimine. No analysis or other proof of structure was given for XVIII.



VIII



XVIII



CXXXIV

Contributions from This Laboratory

Removal of the phthaloyl group from phthaloylglycylethylenimine (VIII), either with the usual hydrazine treatment (103) or the unusual phenylhydrazine treatment (104) might produce CXXXV, a true example of type IV compounds. Neither the hydrazine (in ethanol or non-polar solvents) nor the phenylhydrazine methods were successful. Samples of VIII were prepared and submitted for anticancer screening. In

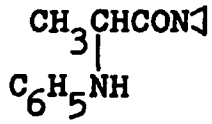
addition, an improved procedure for the preparation of phthaloylglycyl chloride was developed (see Experimental Section).

$H_2NCH_2CON\triangleleft$

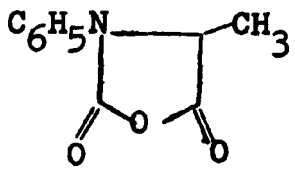
CXXXV

Repetition of Peterson and Gauss' procedure for the synthesis of XVIII did not lead to the results described by them. However, when chloroform was used as the solvent instead of methanol, a material was obtained which agreed in melting point, which possessed an appropriate infra-red spectrum, and which gave a correct elemental analysis. Samples of XVIII were submitted for anticancer screening.

A sequence similar to that described above was used to obtain CXXXVI and CXXXVII. The N-phenylalanine used to prepare CXXXVII had not previously been prepared from α-chloropropionic acid and aniline. N-Phenylalanine-N-carboxyanhydride, CXXXVII, is not known in the literature. A sample of it was submitted for anticancer screening because N-carboxyanhydrides can be polymerized into synthetic proteins (even at room temperature, with a small amount of water), and also because such proteins have shown some surprising results against various experimental tumors (105). Finally, reaction of CXXXVII with ethyl-enimine evolved carbon dioxide and produced CXXXVI, which was submitted for anticancer screening.



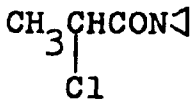
CXXXVI



CXXXVII

This type of sequence is probably limited to N-phenyl-α-amino acid types, due to the greatly increased basicity (and thus ability for further reaction) of aliphatic amino groups.

Some of the difficulties inherent in amino acid chemistry might be obviated if α-chloro compounds were used, leaving transformation of the chloro group to an amino group for a late stage. With this in mind, α-chloropropionic acid was reacted with ethylenimine in the presence of dicyclohexyl-carbodiimide, and CXXXVIII was isolated as a colorless liquid.



CXXXVIII

This material is stable for several weeks when stored in the freezer under nitrogen. Samples were prepared for anticancer screening, but were not submitted. Attempts to convert this compound to the α-amino compound were unsuccessful.

Many other approaches were made to this α-aminoethyl-enamide problem, and all were unsuccessful. Protective groups which were used included the carbobenzoxy, benzoyl, phthaloyl, and trifluoracetyl groups; methods of forming the amide bond included the acid chloride method, use of dicyclo-

hexylcarbodiimide, and the phthaloylglutamic anhydride reaction. Reaction of glycine ester with ethylenimine was tried, as well as several reactions in the α-chloro series, including reactions with ammonia, ethylenimine, and bis-(β-chloroethyl)amine.

Indole and Indole-type Nitrogen Mustards

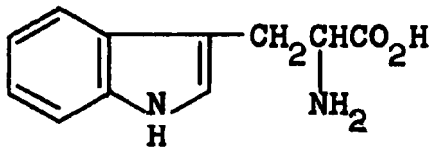
Biochemical Aspects

Background. The rationale for much of the drug design in cancer chemotherapy has been interference with reactions essential for cell duplication, regardless of whether such duplication is of normal cells or of cancerous cells. The success of this approach, to the extent that it has been successful, appears to be a result of the greater rate of growth of tumors, or of some sort of greater inherent susceptibility of a tumor system to a drug. The failure of this approach, to the extent that it has failed, is often due to drug toxicity to those normal tissues which also proliferate rapidly.

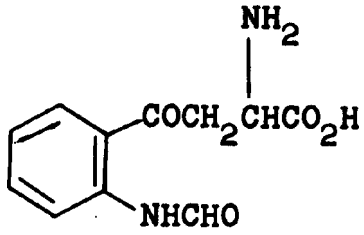
Almost no new abilities of cancerous cells, as compared with normal cells, have been discovered. However, enzyme levels vary considerably between cancerous cells and many types of normal cells. Some cancerous tissues have higher levels of certain enzymes, but a much more common situation is a considerable decrease or even complete loss of certain enzymes in various cancerous cells. This decrease or loss

may put the cancerous cell at a tremendous biochemical disadvantage. Thus, it is conceivable that a properly designed, toxic drug might kill cancerous cells, because of their inability to partially or wholly detoxify the drug; while normal cells, with their more complete complement of enzymes, might render the drug harmless. Ideally, all normal tissues should be capable of detoxifying the drug. However, it is unlikely that the drug would be distributed to all normal tissues. In addition, considerably enhanced drug action still could result if only a few normal tissues (for example, the rapidly proliferating ones) could detoxify the drug. This approach to anticancer drug design is almost untried; its importance has been commented upon by Delmonte and Jukes (106).

The enzyme "tryptophan pyrrolase" catalyzes the conversion of tryptophan (CXXXIX) to N-formylkynurenine (CXL). This enzyme has been the subject of a review (107). Other ways in which tryptophan is metabolized include its incorporation into protein, its degradation to 5-hydroxy-tryptophan (and serotonin and 5-hydroxyindoleacetic acid), and its degradation to indole (and indoxyl and indican). Many biochemical routes arise from N-formylkynurenine, including routes to anthranilic acids, quinolines, nicotinic acids, phenoxazine pigments, etc.



CXXXIX



CXL

Claudatus and Ginori (108) found that tryptophan pyrrolase activity was absent or quite low in a rat and a mouse heptoma. Wood, Rivlin, and Knox (109) showed that tryptophan pyrrolase activity was depressed during the period of most rapid tumor growth and was followed by an increased level in the terminal stages of the disease. Knox (107) writes:

> "This suggests that tryptophan is at first conserved for tissue and tumor growth, and later that more must be degraded to keep pace with the dissolutions of tissue and tumor occurring in the terminal stages."

Udenfriend, Weissbach, and Sjoerdsma (110) found that a patient with a malignant carcinoid tumor metabolized as much as 60% of the tryptophan via the serotonin pathway; in the normal human this value is about 1%. Barajon, Paulet, and Flandre (111) confirmed this observation. Cole and Matthews (112) treated a patient having a metastatic carcinoid tumor with C^{14}-tryptophan, and after removal of the tumor found that the tryptophan had localized in the tumor. In the previously cited paper Udenfriend, Weissbach, and Sjoerdsma, conclude:

"It is apparent that the carcinoid tumor

> is parasitic upon the tryptophan stores of the patient, and, as a result, less of this amino acid may be available for formation of other metabolites, such as protein and niacin. Weight loss and hypoproteinemia are common features of this condition, and pellagra has been reported in several cases. The complex manifestations of this disorder may be related to both a serotonin excess and a tryptophan deficiency."

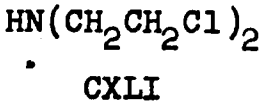
Based upon the preceding discussion, there is reason to hope that tryptophan pyrrolase (or one of the many enzymes concerned with further degradation in normal cells) will be able to detoxify an indole-like or tryptophan-like nitrogen mustard, while the toxic properties of the mustard remain undiminished in the tumor system. In addition, an indole nitrogen mustard may be preferentially taken up by the tumor, as was the labeled tryptophan in Cole and Matthews' experiment.

Organic Chemistry Aspects

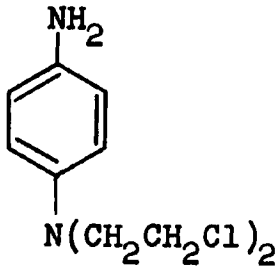
Problems and Approaches to the Synthesis of Special Nitrogen Mustards. Apart from biochemical considerations, an investigation of nitrogen mustards prepared from indole derivatives presents some interest from a purely organic point of view. At the time research for this treatise was initiated no indole nitrogen mustards had been reported and only one indole-like mustard (LXVI) was known.

The preparation of alkylating agents is by no means a simple matter. Often it is necessary to attempt four or five different preparations in order to be successful in one. In addition to difficulties inherent in introducing

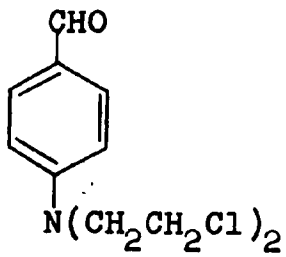
additional functional groups into a molecule, complications may be due to the ability of many nitrogen mustards to alkylate various substrates and intermediates under very mild conditions. This, coupled with the notorious sensitivity of some indoles to acid and other conditions, indicates that special approaches to the synthesis of indole nitrogen mustards must be taken. Thus, the usual procedure of chlorinating a diol to obtain the mustard is not an attractive prospect in the indole series. More desirable would be the introduction of a preformed nitrogen mustard into the indole molecule as the last step of the synthesis. Several such preformed nitrogen mustards, which also contain additional reactive functional groups, are known. Among others, bis(β-chloroethyl)amine (CXLI), p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (CXLII), p-[N,N-bis(2-chloroethyl)amino]aniline (CXLIII), and 1,1-bis(2'-chloroethyl)hydrazine (CXLIV) may be mentioned.



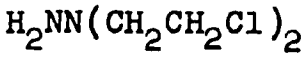
CXLI



CXLIII



CXLII



CXLIV

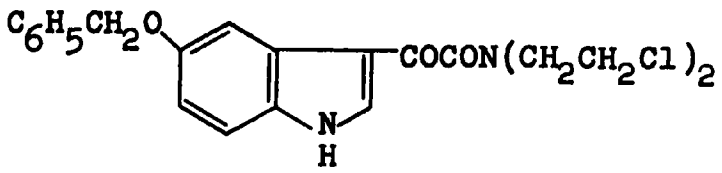
Background. Many amide nitrogen mustards are known. Ross and Wilson (113) hoped this type would be transported into the cell in the amide form, where the amide could be hydrolyzed to release bis(2-chloroethyl)amine. However, they discovered that even on storage, amide nitrogen mustards (CXLV) take up a mole of water, and rearrange to esters (CXLVI).

$RCON(CH_2CH_2Cl)_2$ $RCO_2CH_2CH_2NHCH_2CH_2Cl \cdot HCl$

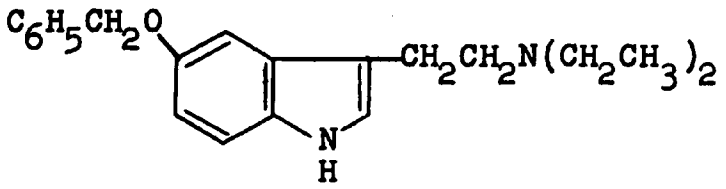
CXLV CXLVI

Because of this facile rearrangement, they concluded that enzymatic release of the bis(2-chloroethyl)amine seemed unlikely. Recently, Pettit, Baumann, and Rangammal (114) reported good anticancer activity for a certain amide nitrogen mustard.

Ross, Warwick, and Roberts (33) reduced molecules containing nitrogen mustard groups with stannous chloride, palladium, or Raney nickel, all without damage to the nitrogen mustard function. Kuwada (27) reduced several amide nitrogen mustards with lithium aluminum hydride, again without replacement of the nitrogen mustard chlorine atoms. However, Kondo, Kataoka, and Dodo (61) failed to obtain a nitrogen mustard from lithium aluminum hydride treatment of XLVIII; on the contrary, they actually isolated some of the reduced, dechlorinated product (CXLVII).



XLVIII

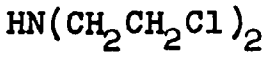


CXLVII

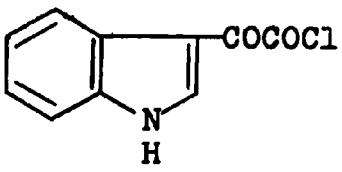
Historical developments in the field of indole-like mustards have been reviewed in the first section of this treatise.

Contributions from This Laboratory. In accordance with the guiding principles developed above for the synthesis of special nitrogen mustards, the preparation of several indole and indole-like nitrogen mustards was attempted.

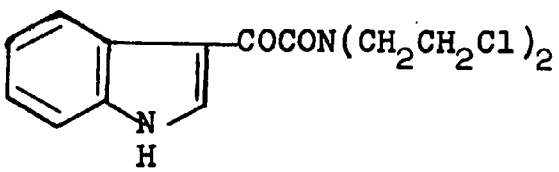
If CXLI acts as an ordinary secondary amine, it should be possible to obtain the amide LVI from the acid chloride CXLVIII. The desired amide LVI was obtained in this manner in 54% yield (after crystallization).



CXLI



CXLVIII

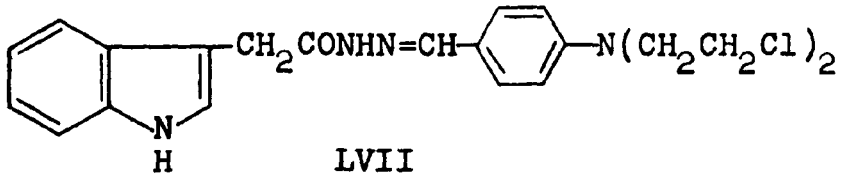


LVI

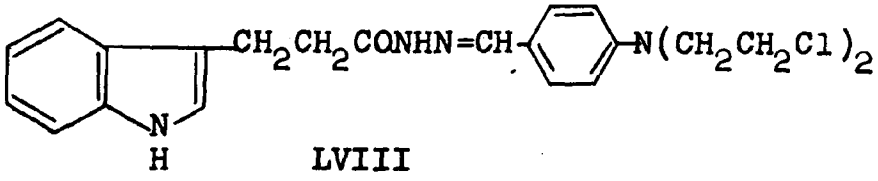
During the early work on the indole amide mustard LVI a very small amount of water soluble, benzene insoluble material was isolated. It is likely that this corresponds to Ross and Wilson's rearranged-type compound CXLVI. This material was not obtained on subsequent larger runs.

Early attempts to reduce the indole amide mustard LVI with lithium aluminum hydride led to intractable products, and in view of Kondo, Kataoka, and Dodo's results, the reaction was not pursued further.

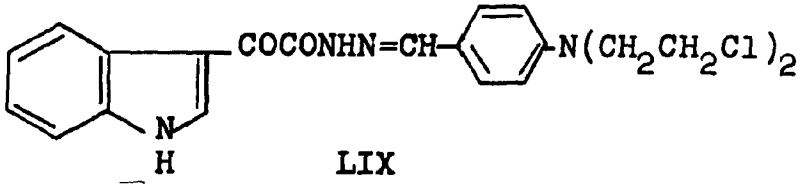
Various hydrazides have been condensed with p-[N,N-bis-(2-chloroethyl)amino]benzaldehyde (CXLII). This approach was used to prepare LVII-LIX (64) and LXII from the corresponding, known hydrazides. In some cases the products were isolated directly from the reaction mixture in analytically pure form.



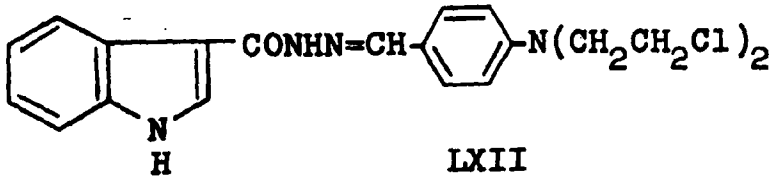
LVII



LVIII

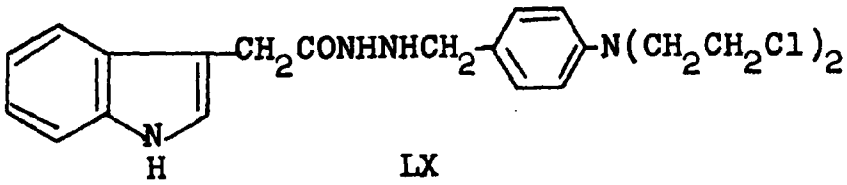


LIX



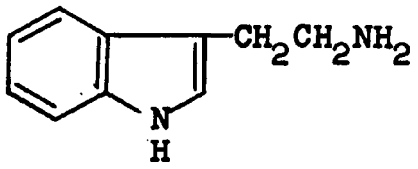
LXII

Lithium aluminum hydride treatment of LVII produced LX in as high as 42% yield (after crystallization) (64). The inertness of hydrazide carbonyl groups to lithium aluminum hydride, under various conditions, has been discussed by Hinman (115).

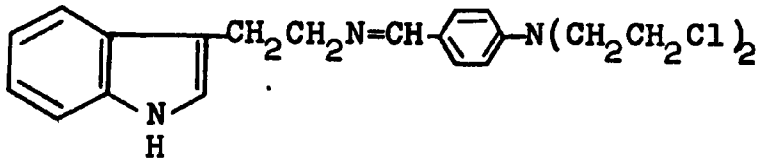


LX

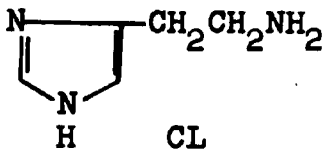
Finally, reaction of tryptamine (CXLIX) with p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (CXLII) produced the corresponding Schiff base (LXI) (64). It is known (116) that Schiff bases derived from aliphatic amines show a greater tendency toward polymerization and subsequent reactions than do Schiff bases derived from aromatic amines. Considerable care was required to obtain LXI. As described later the corresponding reaction with histamine (CL) was unsuccessful. Good anticancer activity has been reported (28,117,118) for certain Schiff base nitrogen mustards. Lithium aluminum hydride reduction of LXI was unsuccessful.



CXLIX



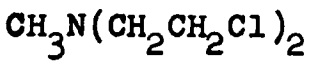
LXI



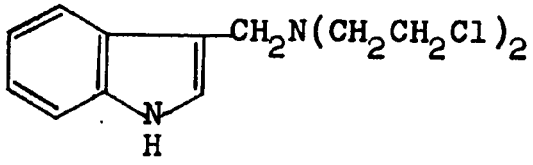
CL

All seven of the indole nitrogen mustards prepared in connection with this treatise were submitted for anticancer screening.

In view of the well-known anticancer properties of N-methyl-bis(2-chloroethyl)amine CLI, it would be desirable to obtain the corresponding nitrogen mustard in which a hydrogen atom from the methyl group of CLI had (hypothetically) been replaced by the indole residue, giving CLII.



CLI



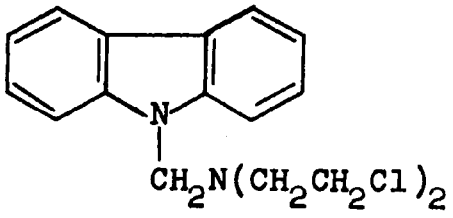
CLII

Many compounds of the general type of CLII have been prepared via the Mannich reaction, using indole, formaldehyde, and various amines. However, it should be pointed out that this reaction is extremely specific in its requirements. Early attempts by various workers to react indole, formaldehyde,

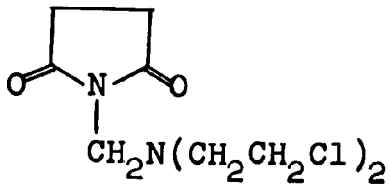
and dimethylamine were completely unsuccessful until it was discovered that the use of glacial acetic acid as solvent allowed the reaction to proceed. Attempts in this laboratory to prepare CLII under almost every conceivable condition were unsuccessful. On the rare occasions that solids were obtained, they proved to be very sensitive to light, and could not be crystallized or otherwise purified. Bis(2-chloroethyl)amine (as various salts) was often recovered. The recently reported (119) procedures involving bis(2-chloroethyl)amine in the Mannich reaction under strongly acidic and strongly basic conditions might be tried; solvent systems involving water and also amine exchange reactions might be tried.

Mannich reactions with bis(2-chloroethyl)amine and skatole, 3-indolemethyl ketone, and 3-indoleacetamide were unsuccessful, as were attempts to repeat the published (120) reaction of indole, formaldehyde, and diethanolamine. Other unsuccessful reactions included treatment of tryptophol and tryptamine with N-chloroformylbis(2-chloroethyl)amine; reaction of 3-indolecarboxylic and 3-indoleacetic acid with bis(2-chloroethyl)amine and dicyclohexylcarbodiimide, reaction of 3-indolecarbonyl and 3-indoleacetyl chloride with bis(2-chloroethyl)amine; reaction of 3-isocyanatomethylindole with bis(2-chloroethyl)amine; and reaction of both tryptamine hydrochloride and p-[N,N-bis(2-chloroethyl)-amino]benzaldehyde, and the Schiff base LXI with hydrogen chloride, in order to obtain the corresponding β-carboline.

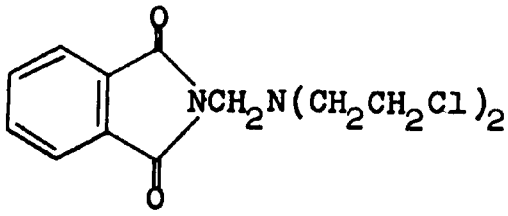
During the course of this work, four nitrogen mustards structurally related to the indole system were prepared and submitted for anticancer screening (64). The four, LXVII-LXX, resulted from the Mannich reaction of bis(2-chloroethyl)amine, formaldehyde, and carbazole, succinimide, phthalimide, and isatin in non-polar organic solvents. Pettit and Settepani (68) concurrently obtained LXIX-LXXII from phthalimide, (and N-hydroxymethylenephthalimide), isatin, 2-oxindoline-3-thiosemicarbazone, and saccharin, using an ethanol-formalin mixture as solvent.



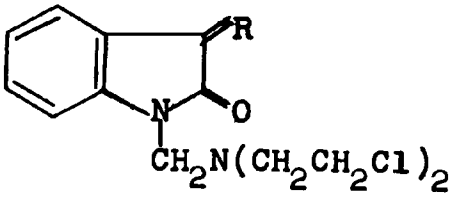
LXVII



LXVIII

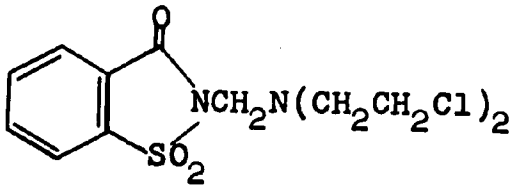


LXIX



LXX R = O

LXXI R = $NNHCSNH_2$



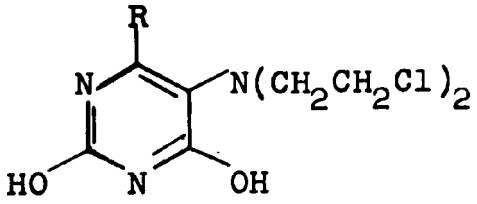
LXXII

Unsuccessful Mannich reactions with bis(2-chloroethyl)-amine included those with benzimidazole, 2-methylbenzimidazole, 2-acetamidothiazole, phthalhydrazide, pyrrole, and nicotinamide. The books by Hellman and Opitz (121) and by Reichert (122) were of considerable value in the literature searches.

Pyrimidine Nitrogen Mustards

Background

Both "Dopan" (LXXIV) and the "Upjohn Uracil Mustard" (LXXV) have received widespread testing and study, and interest in them has led to further work in the pyrimidine series.

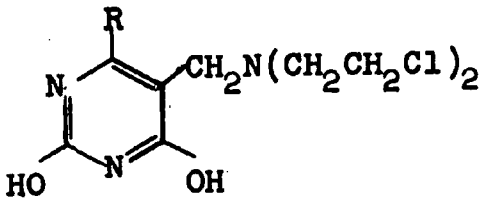


LXXIV R = CH_3

LXXV R = H

Contributions from This Laboratory

Because of the greatly increased chemical reactivity of an aliphatic nitrogen mustard compared with that of an aromatic nitrogen mustard, it was desirable to prepare XCIV and XCV. From another point of view, these compounds represent a (hypothetical) substitution of a uracil residue for a hydrogen atom in the methyl group of the anticancer drug N-methylbis(2-chloroethyl)amine (CLI).



$CH_3N(CH_2CH_2Cl)_2$

CLI

XCIV R = H

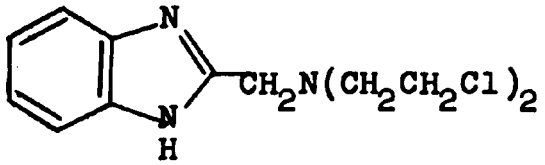
XCV R = CH_3

Both XCIV and XCV have been prepared and submitted for anticancer screening as part of this treatise (38). They resulted from the Mannich reaction of bis(2-chloroethyl)amine, formaldehyde, and uracil or 6-methyluracil in an organic solvent. At about the same time Farkas and Sorm (78) obtained XCIV from bis(2-chloroethyl)amine and 5-chloromethyluracil; and Ross, Lee, Schelstraete, Goodman, and Baker (79) obtained XCIV by chlorination of the diol.

Imidazole and Pteridine Nitrogen Mustards

Background

Even with preparation of the indole nitrogen mustards, there are still two biochemically important heterocyclic systems into which a nitrogen mustard function had not been incorporated. Despite the importance of imidazoles (histidine, histamine, a certain intermediate in purine biosynthesis, etc.), and despite the interest shown at one time in benzimidazole nitrogen mustards of the general type CXXVIII, no imidazole nitrogen mustards have been reported in the literature.

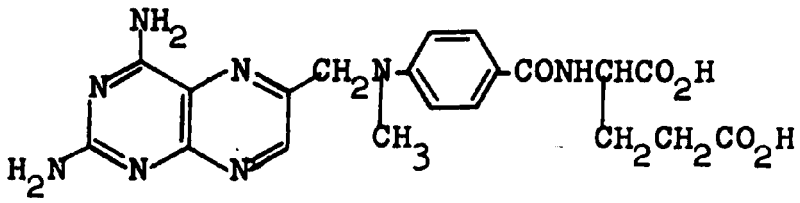


CXXVIII

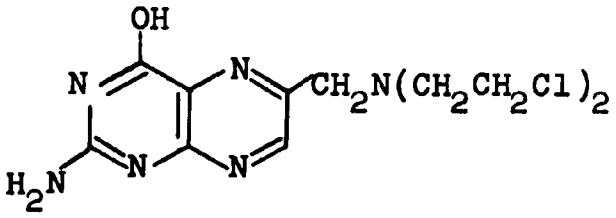
Biological rationales for various imidazole nitrogen mustards might be developed from the following observations. 5-Diazoimidazole-4-carboxamide has anticancer activity (123,124). Inactivation of a certain enzyme was the result of the action of methylbis(2-chloroethyl)amine on histidine and mercapto groups (125). Histamine and a certain antihistamine both inhibit the Ehrlich tumor in mice (126). Histamine present at nerve ends can be fixed by carcinogenic compounds; since histamine is considered to act as a regulator of cell differentiation, this fixation might be responsible for the loss of specificity in cancer growth (127). Various anticancer agents (with one exception, under special conditions), including one nitrogen mustard, have no influence on 4-aminoimidazole-5-carboxamide as a purine precursor (128). A review has been written on histamine and induced cancer (129). The incorporation of labeled formate into nucleic acid proteins in the Ehrlich ascites tumor is greatly increased by 4-aminoimidazole-5-carboxamide and its riboside (130). 4-Aminoimidazole-5-carboxamide partially blocks tumor inhibition of DON and azaserine in mouse sarcoma 180 (131). The enzyme histidase is lost or reduced in the Novikoff hepatoma (11, p. 111). Other pertinent

observations may be found in the earlier literature. Also, the antihistamine activity of a large number of compounds is reported throughout the literature.

The partial successes of Amethopterin (CXXIX) against choriocarcinoma and acute childhood leukemia provide ample justification for the investigation of pteridine nitrogen mustards. Only one example of this type has been reported in the literature (CXXX) (99). Many reviews have been written on folic acid antagonists in general; one of the best reviews is by Martin (132).



CXXIX

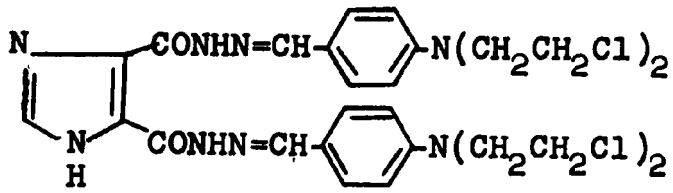


CXXX

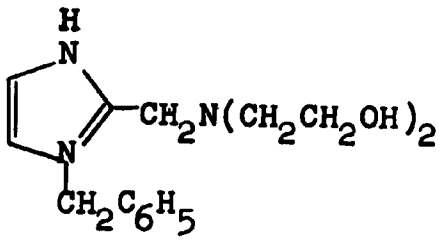
Contributions from This Laboratory

The imidazole nitrogen mustard CLIII was prepared from the corresponding dihydrazide and p-[N,N-bis(2-chloroethyl)-amino]benzaldehyde, and submitted for anticancer screening as part of this treatise. The diol CLIV was obtained by alkylation of diethanolamine. Removal of the benzyl group

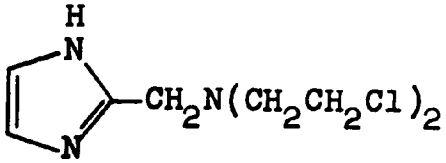
with sodium and liquid ammonia, followed by chlorination, should give CLV. This imidazole nitrogen mustard is related to the benzimidazole nitrogen mustard CXXVIII.



CLIII

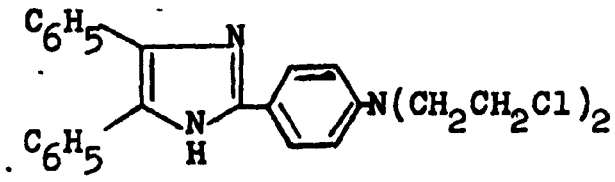


CLIV

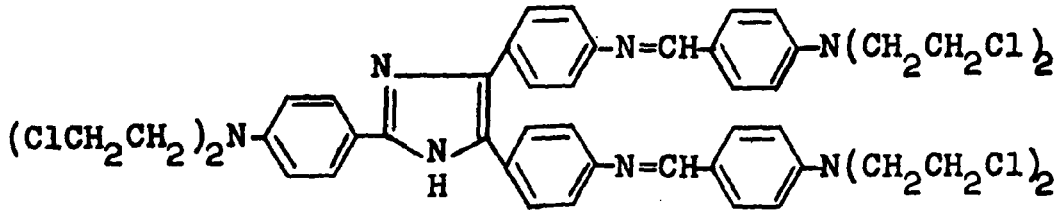


CLV

The reaction of histamine (CL) with p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (CXLII) was unsuccessful, as were the reactions of this aldehyde with ammonium acetate and the appropriate benzil to form CLVI and CLVII (see below for the preparation of the substituted benzil used in the attempt to prepare CLVII).

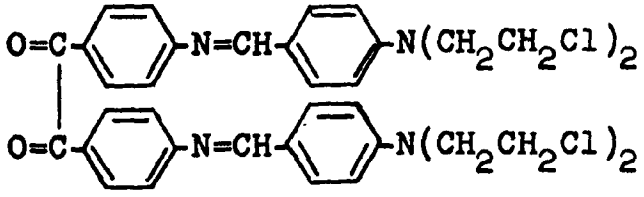


CLVI



CLVII

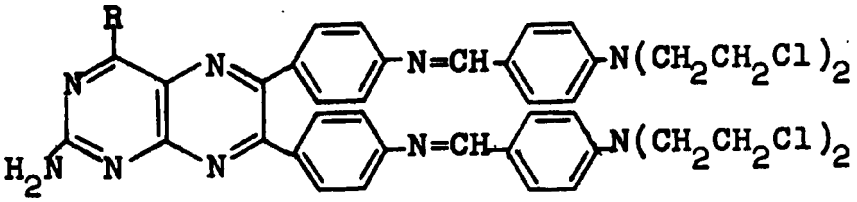
One of the usual approaches to a pteridine synthesis involves reaction of an α-dicarbonyl component with a 4,5-diaminopyrimidine. Since in general, reactions of pteridines, once they are formed, are rather difficult, it was considered best to leave formation of the pteridine nucleus until the last step. For this reason, the α-dicarbonyl nitrogen mustard CLVIII was prepared (and submitted for anticancer screening), using 4,4'-diaminobenzil and p-[N,N-bis(2-chloroethyl)amino]benzaldehyde.



CLVIII

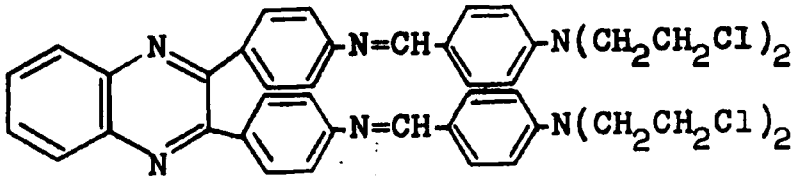
Neither CLIX nor CLX could be obtained from CLVIII and the appropriate 4,5-diaminopyrimidine. One elemental analysis of CLIX indicated that the compound had formed,

but it could not be purified sufficiently. Even the quinoxaline CLXI did not result from CLVIII and o-phenylenediamine.



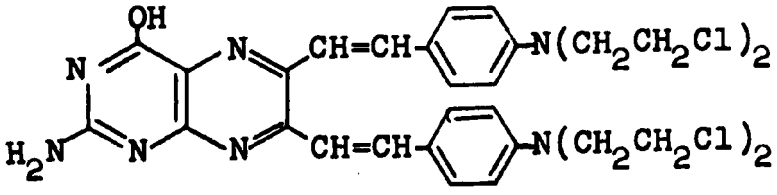
CLIX R = OH

CLX R = NH_2



CLXI

Albert (133) states that methyl groups in the 6- or 7-position of the pteridine ring should react with aldehydes, although 4,7-dihydroxy-6-methylpteridine did not. When 2-amino-4-hydroxy-6,7-dimethylpteridine was reacted with p-[N,N-bis(2-chloroethyl)amino]benzaldehyde in an attempt to prepare CLXII, only tars resulted. There is some indication (134) that methyl groups attached to a pteridine ring exist in the methylene form, produced by transfer of a hydrogen atom from the methyl group to a ring nitrogen.



CLXII

EXPERIMENTAL[a,b]

Phthaloylglycine

Phthaloylglycine was prepared according to the general procedure of Sheehan, Chapman, and Roth (103). A second crystallization from methanol-water raised the m.p. to 188-189°. Lit. (135) m.p. 191-192°.

Phthaloylglycyl Chloride

The procedure of Sheehan and Frank (136) was modified by using thionyl chloride instead of phosphorus pentachloride. Phthaloylglycine (25.00 g., 0.122 mole) was ground with a mortar and pestle, and was then refluxed for 40-50 minutes with thionyl chloride (50 ml.; purified by refluxing overnight with sulfur, distilling, and fractionating through a fifteen plate, packed column). The solution was taken to dryness (water aspirator) and was crystallized from benzene (70 ml.)-petroleum ether (435 ml., 40-60°) to give 22.62 g. (0.101 mole, 83%) of colorless needles, m.p. 87-89°. Lit. (136) m.p. 83-85°.

N-[(1-Aziridinylcarbonyl)methyl]phthalimide (VIII)

This preparation was carried out according to the procedure of Fles and Markovac-Prpic (137), except that

---

[a]All melting points are corrected, and all boiling points are uncorrected, unless noted.

[b]Microanalyses were done by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

benzene-petroleum ether (40-60°) was used for the crystallization. After two crystallizations, the product melted at 116.5-117.5°. Lit. (137) m.p. 116°.

N-Phenylglycine

N-Phenylglycine was prepared according to de Mouilpied (138). The crude product, used in the next step, melted at 108.5-111.5. Beilstein gives melting points varying from 125° to 128°.

N-Phenylglycine-N-carboxyanhydride (CXXXIV)

This preparation was carried out according to the general procedure described by Bamford, Elliott, and Hanby (139, p. 48), using chlorobenzene as solvent (50). The product was obtained in 96% yield, and melted at 135-137°. Sublimation, which was more satisfactory than crystallization from ethyl acetate or ethyl acetate-petroleum ether, lowered the melting point to 130-131°. Lit. (139) m.p. 139°.

1-(N-Phenylglycine)ethylenamide (XVIII)

The use of crude N-phenylglycine-N-carboxyanhydride and methanol as solvent, as described in the literature (50), did not produce the desired product. The reaction was also unsuccessful when sublimed N-phenylglycine-N-carboxyanhydride and methanol were used. Finally, ethylenimine (1.80 ml., 0.035 mole) was stirred in reagent chloroform (3.90 ml., dried over calcium chloride), and sublimed N-phenylglycine-N-carboxyanhydride (5.31 g., 0.030 mole) was added in

portions over 40 minutes (gas evolved). At first an ice-bath was used, but the temperature was gradually raised to room temperature during the 40 minutes, and then gradually raised to 34° during 10 minutes. The reaction mixture was chilled overnight in the freezer to give 3.74 g. (0.021 mole, 71%) of product, m.p. 69-71°. A second crop (0.46 g., m.p. 65-68°) and a third crop (0.21 g., m.p. 66-68°) separated from the mother liquor; total, 4.41 g. (84%). Crystallization from chloroform (the solution should not be heated above 30°) gave a rather low yield of material, m.p. 72-74°. The analytical sample was crystallized three times from chloroform, m.p. 71-73°.

Anal. Calcd. for $C_{10}H_{12}N_2O$: C, 68.16%; H, 6.86%; N, 15.90%.
Found: C, 68.02%; H, 6.88%; N, 15.81%.

Gauss and Petersen (50) report m.p. 69.5-70°, and 69% yield (no analysis given).

N-Phenylalanine

α-Chloropropionic acid (86.82 g., 0.800 mole), reagent aniline (149.00 g., 1.600 mole), and water (400 ml.) were heated on the steam bath for 2 hours, with occasional shaking. The mixture was then poured into a beaker, cooled, and filtered. The precipitate was dissolved in an excess of 10% sodium hydroxide solution, and filtered through a medium sintered glass funnel. Acidification to pH 4 with concentrated hydrochloric acid gave, after extensive drying in vacuum, 103 g. (78%) of material, m.p. 157.5-159.5°.

Beilstein gives m.p. 163°, 162°, and 157°. This material was satisfactory for the next step.

N-Phenylalanine-N-carboxyanhydride (CXXXVII)

N-Phenylalanine (15.00 g., 0.0908 mole) was suspended in tetrahydrofuran (150 ml., distilled from lithium aluminum hydride), and a fairly rapid stream of phosgene was passed in for 100-160 minutes, keeping the temperature below 40° with a water-bath. (A sodium hydroxide trap is best for the phosgene). The volume of the solution increased considerably during this time. The dark red solution was filtered through paper, and the filtrate was taken to dryness (water aspirator). The residue did not solidify at this point unless dried in vacuum, but it could be used directly for sublimation. After sublimation in high vacuum, just below the melting point, 10.37 g. (59%) of colorless crystals were obtained. The analytical sample was sublimed twice, m.p. 99-100°.

Anal. Calcd. for $C_{10}H_9NO_3$: C, 62.82%; H, 4.75%; N, 7.33%.
Found: C, 62.80%; H, 4.78%; N, 7.40%.

1-(N-Phenylalanine)ethylenamide (CXXXVI)

Ethylenimine (1.42 ml., 0.0275 mole) was stirred in reagent chloroform (4.0 ml., dried over calcium chloride), and sublimed N-phenylalanine-N-carboxyanhydride (4.76 g., 0.025 mole) was added in portions during 30 minutes (gas evolved). At first an ice-bath was used, but after the addition was complete, the temperature was raised to 30° in order to dissolve all of the solid. Most of the solvent was

taken off at the vacuum pump; crystallization was then allowed to proceed in the freezer, giving 3.50 g. (74%) of product, m.p. 77.5-78.5° (put in oil-bath at 66°). Two crystallizations from chloroform (no heating) produced an analytical sample, m.p. 78.5-80°.

Anal. Calcd. for $C_{11}H_{14}N_2O$: C, 69.44%; H, 7.42%; N, 14.73%.
Found: C, 69.22%; H, 7.45%; N, 14.84%.

1-(2'-Chloropropionyl)ethylenamide (CXXXVIII)

α-Chloropropionic acid (10.85 g., 0.100 mole of Eastman Kodak Co. material) and reagent chloroform (90 ml.) were stirred in an ice-bath. Ethylenimine (5.17 ml., 0.100 mole) in chloroform (40 ml.) was added from one funnel, while dicyclohexylcarbodiimide (20.63 g., 0.100 mole of Eastman Kodak Co. material) in chloroform (30 ml.) was added from a second funnel. The addition was done at such a rate that the temperature did not rise above 9° (30 minutes). Stirring was continued at room temperature for 3.5 hours. Dicyclohexylurea (21.88 g., 98%) was filtered off, and the filtrate was distilled. After removal of the chloroform, 1(2'-chloropropionyl)ethylenimine (6.85 g., 51%) distilled at 42°/0.9 mm. An analytical sample was taken during a second distillation; b.p. 32.8°/0.2 mm.

Anal. Calcd. for C_5H_8ClNO: C, 44.95%; H, 6.04%; N, 10.49%.
Found: C, 44.86%; H, 5.90%; N, 10.30%.

Bis(2-chloroethyl)amine (CXLI)

Bis(2-chloroethyl)amine was prepared by treating the

hydrochloride (140) in water with an excess of 5% sodium hydroxide, extracting three times with ether, washing the combined ether extracts with water, drying them (magnesium sulfate), and removing the ether at the aspirator.

N-[Bis(2-chloroethyl)aminomethyl]isatin (LXX)

Crude isatin (from National Aniline Division of Allied Chemical Corp.) was crystallized twice from water (Norite). Then isatin (14.71 g., 0.100 mole), and bis(2-chloroethyl)-amine (from 17.95 g., 0.100 mole, of the hydrochloride) were refluxed in reagent benzene (1250 ml.) for 25 minutes. The mixture was filtered hot, concentrated (aspirator) to one-third its original volume, treated with magnesium sulfate and Norite, filtered, and the filtrate taken to dryness (aspirator). Crystallization from chloroform-petroleum ether (40-60°), after removal of two small, high-melting crops, gave 15.40 g. (0.0511 mole, 51%) of material, m.p. 78-80°. The analytical sample was crystallized once from ether and twice from chloroform-petroleum ether (40-60°); m.p. 82.5-83°. A better solvent system for the purification was ethyl acetate-petroleum ether (60-75°).

Anal. Calcd. for $C_{13}H_{14}Cl_2N_2O_2$: C, 51.84%; H, 4.69%; N, 9.30%.
Found: C, 51.97%; H, 4.72%; N, 9.32%.

N-[Bis(2-chloroethyl)aminomethyl]phthalimide (LXIX)

Phthalimide (7.36 g., 0.0500 mole), formalin (4.5 ml. of 37%, about 0.055 mole), and bis(2-chloroethyl)amine (from

8.93 g., 0.0500 mole, of the hydrochloride) were refluxed in benzene (750 ml.) for 25 minutes. The hot solution was decanted away from the small water layer, filtered, and taken to dryness (aspirator). The resulting residue was extracted with portions of boiling ether (175 ml. total), and filtered; the insoluble remainder (0.36 g.) was identified as phthalimide. The ether filtrate was treated with Norite, filtered, and petroleum ether (40-60°; 250 ml.) added, and the flask kept in the freezer overnight to give 10.11 g. (0.0336 mole, 67%) of product, m.p. 76-79°. The analytical sample was crystallized twice from ether-petroleum ether (40-60°), but a better solvent system was benzene-petroleum ether (40-60°).

Anal. Calcd. for $C_{13}H_{14}Cl_2N_2O_2$: C, 51.84%; H, 4.69%; N, 9.30%.
Found: C, 51.95%; H, 4.75%; N, 9.40%.

9-[Bis(2'-chloroethyl)aminomethyl]carbazole (LXVII)

Carbazole (16.72 g., 0.100 mole), formalin (9.0 ml. of 37%, about 0.11 mole), and bis(2-chloroethyl)amine (from 17.85 g., 0.100 mole, of the hydrochloride) were refluxed in benzene (500 ml.) for 30 minutes. The solvent was removed at the aspirator, and the resulting residue was extracted several times with ether. The ether extracts were treated with magnesium sulfate and Norite, saturated at the boiling point with petroleum ether (40-60°), and set in the freezer. This produced 13.41 g. (0.0418 mole, 42%) of colorless crystals and a smaller amount (5.21 g., 0.0162 mole,

16%) of a tan material, which was easily separated by hand. Another crystallization from ether-petroleum ether gave material melting at 52-54°. It is best not to cool below room temperature during the crystallization. The analytical sample was also crystallized twice from ether-petroleum ether (40-60°); m.p. 56-58°.

Anal. Calcd. for $C_{17}H_{18}Cl_2N_2$: C, 63.56%; H, 5.65%, N, 8.72%.
Found: C, 63.55%; H, 5.58%, N, 8.84%.

N-[Bis(2-chloroethyl)aminomethyl]succinimide (LXVIII)

Succinimide (10.90 g., 0.110 mole), formalin (9.9 ml. of 37%, about 0.12 mole) and bis(2-chloroethyl)amine (from 19.64 g., 0.110 mole, of the hydrochloride) were stirred in chloroform (110 ml.) for 45 minutes at room temperature. The chloroform layer was then separated from a small water layer, dried (magnesium sulfate), and concentrated to 60 ml. (aspirator), treated with Norite, saturated at 40° with petroleum ether (40-60°), and allowed to cool to room temperature before setting in the freezer and adding more portions of petroleum ether. This produced 20.41 g. (0.0806 mole, 73%) of product, m.p. 65-68°. The analytical sample was crystallized three times from chloroform-petroleum ether (40-60°), m.p. 69-71°.

Anal. Calcd. for $C_9H_{14}Cl_2N_2O_2$: C, 42.70%; H, 5.58%; N, 11.07%.
Found: C, 42.68%; H, 5.65%; N, 11.12%.

N,N-Bis(2'-chloroethyl)-3-indoleglyoxylamide (LVI)

Bis(2-chloroethyl)amine (from 40.6 g., 0.227 mole, of

the hydrochloride) and triethylamine (31.5 ml., 0.227 mole) were stirred in chloroform (200 ml.) and cooled in an ice-salt mixture. 3-Indoleglyoxalyl chloride (141) (47.08 g., 0.227 mole) was then added at such a rate that the temperature did not rise above 10°. After the addition was complete, stirring and cooling were continued for 15 minutes before washing the mixture with water (three portions, 200 ml. each), treating the chloroform layer with magnesium sulfate and Norite, and saturating it at the boiling point with petroleum ether (60-75°). This resulted in 37.02 g. (0.118 mole, 52%) of product, m.p. 120-122°. Crystallization from ethyl acetate-petroleum ether (60-75°) then gave 29.53 g. of material, m.p. 125-126°. The analytical sample was crystallized first from chloroform-petroleum ether, then from benzene, and then from ethyl acetate-petroleum ether (60-75°) without heating; m.p. 121-122°.

Anal. Calcd. for $C_{14}H_{14}Cl_2N_2O_2$: C, 53.69%; H, 4.51%; N, 8.95%.
Found: C, 53.84%; H, 4.54%; N, 9.01%.

None of the hydrolyzed, rearranged type of product (113) was observed in the larger scale run, but a small amount of bright yellow, water soluble, benzene insoluble material, m.p. 166-168°, was isolated from the smaller (analytical sample) run.

Purification of p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (CXLII)

Crude p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (19)

(100 g.) was dissolved in technical benzene (400 ml.) by warming to 27-30°. The dark purple solution was chromatographed on a 1 3/4" diameter column of Merck alumina (basic-washed, No. 71707). After the 400 ml. of benzene had passed through the column, another 300 ml. of benzene were run through the column, the yellow eluate heated nearly to boiling, and treated with petroleum ether (90-100°, 1200 ml., no cloudiness). Long, pale yellow needles (77.16 g., 77%), m.p. 83-84° were obtained. Lit. (19) m.p., 85-88° from ethanol. On a 90.00 g. run, pale green needles (80%), m.p. 87-89°, were produced, and on a 10.00 g. run, light purple needles (55%), m.p. 81-82°, resulted (lower boiling fractions of petroleum ether were used, producing cloudiness at the boiling point).

3-Indoleacet[4'-bis(2"-chloroethyl)amino]benzylidene-hydrazide (LVII)

Indole-3-acethydrazide (142) was prepared from indole-3-acetic acid ethyl ester (143).

Indole-3-acethydrazide (7.57 g., 0.0400 mole) and p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (9.85 g., 0.0400 mole) were refluxed in absolute ethanol (740 ml.) for 3 hours, the contents of the flask cooled, the precipitate collected and washed thoroughly with absolute ethanol, and dried to give 15.19 g. (0.0364 mole, 92%) of cream-colored material, m.p. 193-194°. Analytical precautions had been taken in preparing this batch, and a portion of it was

submitted directly for analysis.

Anal. Calcd. for $C_{21}H_{22}Cl_2N_4O$: C, 60.43%; H, 5.31%; N, 13.43%.
Found: C, 60.42%; H, 5.33%; N, 13.50%.

Crystallization from nitromethane lowered the melting point to 187-188°; crystallization from N,N-dimethylformamide-ethanol was unsatisfactory.

3-Indoleglyoxyl[4'-bis(2"-chloroethyl)amino]benzylidene-hydrazide (LIX)

3-Indoleglyoxylhydrazide (144) was prepared from indole-3-glyoxylic acid ethyl ester, which in turn was prepared from 3-indoleglyoxalyl chloride (141). The latter reaction mixture was stirred 14 hours at room temperature to give 87% of the ester. One group of workers (145) allowed the reaction to proceed for 60 hours, and obtained about 90% yield. Another group (146) ran the reaction overnight at 5°, and got 55%.

3-Indoleglyoxylhydrazide (10.16 g., 0.0500 mole) and p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (12.31 g., 0.0500 mole) were refluxed in absolute ethanol (1000 ml.) for 3 hours, the contents of the flask cooled, and the precipitate collected, washed wtih absolute ethanol, and dried to give 19.93 g. (0.0462 mole, 92%) of bright yellow material, m.p. 221-222°. The smaller, analytical sample was prepared in the same way and isolated directly from the reaction mixture, m.p. 220-221°.

Anal. Calcd. for $C_{21}H_{20}Cl_2N_4O_2$: C, 58.47%; H, 4.67%; N, 12.99%.
Found: C, 58.52%; H, 4.71%; N, 12.95%.

The product crystallized as bright yellow needles from nitromethane, but the melting point was lowered to 216-217°.

1-(3'-Indoleacet)-2-[4"-bis(β-chloroethyl)amino]benzylhydrazine (LX)

3-Indoleacet[4'-bis(2"-chloroethyl)amino]benzylidenehydrazide (27.10 g., 0.0646 mole) was added in portions to a stirred mixture of lithium aluminum hydride (7.74 g., 0.194 mole as 95% pure) in tetrahydrofuran (300 ml., distilled from lithium aluminum hydride), using a room temperature water-bath. Stirring was continued for 1 hour after the addition was complete. After careful, dropwise decomposition with water, the solids were filtered off, washed thoroughly with tetrahydrofuran, and then extracted twice with 100 ml. portions of boiling ethyl acetate. The combined tetrahydrofuran and ethyl acetate fractions were treated with magnesium sulfate and Norite, filtered, and the filtrate taken nearly to dryness (aspirator). Crystallization of the residue from ethyl acetate-petroleum ether (60-75°) gave 11.42 g. (0.0273 mole, 42%) of pale orange material, m.p. 148-149°. The analytical sample was crystallized from absolute ethanol-petroleum ether (60-75°), and then from ethyl acetate-petroleum ether (60-75°), m.p. 139-140°.

Anal. Calcd. for $C_{21}H_{24}Cl_2N_4O$: C, 60.14%; H, 5.77%; N, 13.36%.
Found: C, 60.10%; H, 5.71%; N, 13.43%.

β-(3-Indole)propionic Acid

β-(3-Indole)propionic acid may be purchased (Regis Chemical Co. or Eastman Kodak Co.), or much less conveniently, synthesized from the nitrile (147), which is obtained from indole and acrylonitrile (147). The preparation of β-(3-indole)propionic acid from indole and β-propiolactone (148) did not give us satisfactory results.

β-(3-Indole)propionic Acid Ethyl Ester

The esterification of β-(3-indole)propionic acid is reported in the literature (149), but no directions are given for working up to reaction mixture.

β-(3-indole)propionic acid (16.50 g., 0.0872 mole) was refluxed for 3 hours in absolute ethanol (50 ml.) containing concentrated sulfuric acid (2.5 ml.). The alcohol was then removed (aspirator, hot water-bath), excess sodium bicarbonate solution added, the mixture extracted three times with 100 ml. portions of ether, the combined ether layers washed with water, dried (magnesium sulfate), the ether removed (aspirator), and the residue distilled at 173-176° at 0.8 mm. to give 14.16 g. (0.0653 mole, 75%) of a solid, m.p. 37-40° (uncorr.). The literature (149) reported 78% based on 2-carboxyindole-3-propionic acid, b.p. 171° at 4 mm., m.p. 43-45°.

β-(3-Indole)propiohydrazide

The reaction of β-(3-indole)propionic acid methyl ester with hydrazine is reported in the literature (150,151), but no experimental details are given.

β-(3-Indole)propionic acid methyl ester (16.89 g., 0.0779 mole) and 100% hydrazine hydrate (5.84 ml., about 0.120 mole) were stirred in an oil-bath at 140° for 2 hours. Removal of the volatile components (aspirator, hot water-bath) left a solid residue which was crystallized from absolute ethanol-petroleum ether (60-75°) to give 13.04 g. (0.0644 mole, 82%) of small, white needles, m.p. 121-123°. A second crystallization from the same solvent system raised the melting point to 123-124°. A third crystallization, from ethyl acetate, produced material which melted quite sharply at 123-124°. A fourth crystallization, from 95% ethanol, did not change the melting point. An earlier run on a smaller scale was crystallized from water to give long needles, m.p. 132-134°, and also a solidified oil layer, m.p. 125-127°. Ashley and Robinson (151) report a 97% yield and m.p. 140-141° (from ethanol).

β-(3-Indole)propio[4'-bis(2"-chloroethyl)amino]benzylidene-hydrazide (LVIII)

β-(3-Indole)propionic acid hydrazide (8.20 g., 0.0404 mole) and p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (9.94 g., 0.0404 mole) were refluxed in absolute ethanol (200 ml.) for 3 hours, the contents of the flask chilled, and the precipi-

tate collected and washed extensively with absolute ethanol to give 15.79 g. (0.0366 mole, 91%) of a fluffy, colorless solid, m.p. 166-167°. An earlier, smaller run melted at 167-168°, and was submitted directly for analysis.

Anal. Calcd. for $C_{22}H_{24}Cl_2N_4O$: C, 61.25%; H, 5.61%; N, 12.99%.
Found: C, 61.11%; H, 5.74%; N, 12.95%.

Indole-3-carboxylic Acid

The decarbonylation of 3-indoleglyoxalyl chloride followed by hydrolysis of the resulting crude 3-indolecarbonyl chloride (152) was far superior to carbonation of the Grignard reagent (153), or oxidation of indole-3-aldehyde with aqueous permanganate (154) or potassium permanganate in acetone (155), or oxidation with silver oxide (unpublished attempt, in this laboratory).

Indole-3-carboxylic Acid Ethyl Ester

The reaction of ethanol with crude 3-indolecarbonyl chloride (152) gave a product which was quite difficult to purify.

Indole-3-carboxylic Acid Methyl Ester

Various procedures, including esterification of indole-3-carboxylic acid (25), have been used to obtain indole-3-carboxylic acid methyl ester. The procedure described below is a modification of Peterson, Wolf, and Nieman's procedure (152).

Crude indole-3-carbonyl chloride (152) (from 29.29 g.,

0.250 mole, of indole, via 3-indoleglyoxalyl chloride) was added in several portions to reagent methanol (300 ml.). A cold water-bath was used during the addition. After stirring at room temperature for one hour, water (360 ml.) was added to the dark purple solution, and 33.8 g. (0.193 mole, 77% based on indole) of a light purple product were filtered off. Crystallization from methanol (140 ml.)-water (120 ml.) gave 31.7 g. of tan powder, m.p. 134-138°. Lit. (152) m.p. 144-145.6°. Crystallization again from methanol (70 ml.) gave 22.5 g., m.p. 139-141°. A third crystallization from ethyl acetate (64 ml.) gave 16.12 g., m.p. 142-143°.

Indole-3-carboxhydrazide

Reaction of either crude (152) or pure (144) indole-3-carbonyl chloride with hydrazine (144) did not give satisfactory results, nor did reaction of indole-3-carboxylic acid ethyl ester with hydrazine at 45° for 6 hours (156). Kakimoto and Nishie (155) report obtaining the hydrazide from the methyl ester and 70% hydrazine hydrate after 6 hours of reflux on the water-bath, but they give no further details.

Indole-3-carboxylic acid methyl ester (16.12 g., 0.092 mole), 100% hydrazine hydrate (17.5 ml., 0.35 mole), and water (7.4 ml.) were heated at 120° (oil-bath temperature), with stirring, for 2.5 hours. The volatile components were removed (aspirator, hot water-bath), and the residue was taken up in about 700 ml. of 95% ethanol and boiled down to 300 ml. to give 13.00 g. (0.0742 mole, 81%) of almost colorless,

thick needles, m.p. 224-226°. Lit. (155) m.p. 225° (from water).

Indole-3-carbox[4'-bis(2"-chloroethyl)amino]benzylidene-hydrazide (LXII)

Indole-3-carboxhydrazide (7.01 g., 0.040 mole) and p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (9.85 g., 0.040 mole) were refluxed in absolute ethanol (800 ml.) for 3 hours. The volatile materials were removed (aspirator, 45° water-bath), and the residue was crystallized (Norite) from nitromethane (450 ml.) to give 13.52 g. (0.0336 mole, 84%) of orange crystals, m.p. 188-189° (dec.) (melting point tube put in at 177°). The analytical sample was also crystallized once from nitromethane, m.p. 187-188° (put in at 177.5°).

Anal. Calcd. for $C_{20}H_{20}Cl_2N_4O$: C, 59.56%; H, 5.00%; N, 13.89%.
Found: C, 59.36%; H, 5.11%; N, 13.77%.

N-[4-(Bis((2'-chloroethyl))amino)]benzylidenetryptamine (LXI)

Tryptamine hydrochloride (Regis Chemical Co.; 9.83 g., 0.0500 mole) was washed with water into a separatory funnel containing ether. Excess 5% sodium hydroxide was then added (the reverse order of addition ultimately results in a yellow oil instead of crystalline tryptamine), the contents shaken extensively, and the layers separated. After two more extractions with ether, the combined extracts were washed once with water, dried (magnesium sulfate), and concentrated (aspirator) to 500 ml. (Concentration to dryness results in

the white, crystalline tryptamine free base).

p-[N,N-Bis(2-chloroethyl)amino]benzaldehyde (12.31 g., 0.0500 mole) was added to the above solution, which was then warmed slightly and stirred until all the aldehyde dissolved. The solution was then concentrated (aspirator) to 125 ml., and refluxed gently for 60 minutes. Reagent benzene (100 ml.) was added, and the volatile materials removed (aspirator, 40° water-bath); evaporation was repeated twice more with 250 ml. portions of benzene. The resulting yellow oil was taken up in ethyl acetate (45 ml.), treated with Norite, filtered, and treated with petroleum ether (60-75°; 100 ml.) at the boiling point; slight boiling occurred. On cooling, a layer of oil (soon solidifying) separated, in addition to the fine, beige needles usually obtained at this point. The yield was 12.77 g. (0.0328 mole, 66%). This was crystallized again, using less petroleum ether (60-75°). This time larger, somewhat orange crystals were obtained (11.62 g., m.p. 114-115°), which could be ground up to a nearly white powder, and which were identical with the analytical sample in mixed melting point and infrared spectrum. The analytical sample was crystallized only once, from ethyl acetate-petroleum ether (60-75°), m.p. 113-114°.

Anal. Calcd. for $C_{21}H_{23}Cl_2N_3$: C, 64.95%; H, 5.97%; N, 10.82%.

Found: C, 64.98%; H, 5.88%; N, 10.88%.

5-[Bis(2'-chloroethyl)aminomethyl]-6-methyluracil Hydrochloride (XCV)

6-Methyluracil (157) (10.08 g., 0.0800 mole, finely powdered), paraformaldehyde (2.53 g., 0.0800 mole, assuming 95% purity), and bis(β-chloroethyl)amine hydrochloride (14.28 g., 0.0800 mole) were heated rapidly to 90° in N,N-dimethylformamide (200 ml.), with stirring. The solution soon became clear, and shortly thereafter, precipitation started. The reaction was heated at 90 ± 2° for a total of 30 minutes. It was then rapidly cooled to 6°, left in the freezer for four hours, filtered, washed with N,N-dimethylformamide and then ether, and finally dried in high vacuum to constant weight to give 13.72-14.88 g. (0.0434-0.0470 mole, 54-59%, two runs). M.p. 196-199° (dec.).

A volume of 200 ml. of N,N-dimethylformamide is almost the minimum of solvent which will still produce a clear solution. Use of excess paraformaldehyde and bis(β-chloroethyl)amine hydrochloride resulted in a much lower yield. Longer heating (up to 2 hours) resulted in a slightly lower yield. Carrying out the reaction at 80° did not increase the yield. A second, acceptable fraction could not be obtained by allowing the mother liquor to stand, by concentrating the mother liquor, or by diluting it with ether.

Crystallization from methanol and then methanol-ether gave an analytical sample, m.p. 204-205° (dec.). The product from other runs melted as high as 206-206.5° (dec.).

Anal. Calcd. for $C_{10}H_{15}Cl_2N_3O_2 \cdot HCl$:

C, 37.93%; H, 5.09%; N, 13.27%.

Found: C, 38.11%; H, 5.14%; N, 13.28%.

5-[Bis(2'-chloroethyl)aminomethyl]uracil Hydrochloride (XCIV)

Uracil (5.61 g., 0.0500 mole), paraformaldehyde (1.58 g., 0.0500 mole, assuming 95% purity), and bis(β-chloroethyl)-amine hydrochloride (8.93 g., 0.0500 mole) were heated rapidly to 110° in glacial acetic acid (90 ml.), with stirring. After about 15 minutes, a clear orange solution was obtained. The reaction was heated at 110 ± 1° for a total of 2 hours. It was then rapidly cooled, stirred at room temperature for 2 hours, the precipitate filtered off, washed, and dried to give 6.90 g. (0.0228 mole, 46%) of material which turned yellow at 192-197°.

Varying the concentration, temperature, or reaction time all resulted in considerably lower yields. Crystallization often proceeded in rather low yield.

The analytical sample was crystallized three times from methanol-ether, and melted at 210-211°.

Anal. Calcd. for $C_9H_{13}Cl_2N_3O_2 \cdot HCl$:

C, 35.72%; H, 4.66%; N, 13.89%.

Found: C, 35.82%; H, 4.55%; N, 13.80%.

Imidazole-4,5-di[carbox(4'-bis((2"-chloroethyl))amino)-benzylidenehydrazide] (CLIII)

Imidazole-4,5-dicarboxhydrazide was prepared (158) from imidazole-4,5-dicarboxylic acid dimethyl ester.

Imidazole-4,5-dicarboxhydrazide (2.76 g., 0.0150 mole) and p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (7.39 g., 0.0300 mole) were refluxed in absolute ethanol (300 ml.) for 5 hours. After standing overnight in the refrigerator, 8.25 g. (0.0129 mole, 86%) of bright yellow precipitate were collected. M.p. 243-244° (vac.). The analytical sample (1.03 g.) was crystallized once from nitromethane (105 ml.), m.p. 222-222.5° (vac.).

Anal. Calcd. for $C_{27}H_{30}Cl_4N_8O_2$:

C, 50.64%; H, 4.72%; N, 17.50%.

Found: C, 50.67%; H, 4.85%; N, 17.34%.

1-Benzyl-2-[bis(β-hydroxyethyl)aminomethyl]imidazole (CLIV)

To a stirred, refluxing solution of diethanolamine (6.84 g., 0.065 mole) in n-propyl alcohol (15 ml., dried over potassium carbonate and distilled) was added, in small portions, 1-benzyl-2-chloromethylimidazole hydrochloride (1.22 g., 0.005 mole, prepared in five steps (159) from benzylamine and chloroacetal). After refluxing 6 hours, the yellow solution was cooled, made basic with potassium carbonate solution, and extracted with ethyl acetate (3 x 20 ml.), in which wet diethanolamine is insoluble. The combined extracts were washed, dried, taken to dryness (aspirator, hot water-bath), and the residue taken up in absolute ethanol, treated with Norite, excess alcoholic hydrogen chloride added, and the volatile materials again removed in vacuum. The resulting oil was dissolved in

absolute ethanol, and the solution was saturated at the boiling point with ether. After standing overnight in the refrigerator, 1.00 g. (0.00327 mole, 65%) of colorless crystals, m.p. 153-154°, separated. The analytical sample was crystallized again from methanol-ether, and crystallized a third time from absolute ethanol-ether, m.p. 156-157°.

Anal. Calcd. for $C_{15}H_{21}N_3O_2 \cdot 2HCl$:

C, 51.73%; H, 6.66%; N, 12.07%.

Found: C, 51.78%; H, 6.71%; N, 12.18%.

dl-4,4'-Diacetamidobenzoin

The yield obtained by Gee and Harley-Mason (160) was improved by adding water to the reaction (161). Both the white form (formerly supplied by Eastman Kodak Co.) and the yellow form (supplied by Aldrich Chemical Co., Inc.) (162) of p-acetamidobenzaldehyde were satisfactory.

p-Acetamidobenzaldehyde (500.0 g., 3.062 mole), potassium cyanide (50.00 g., 0.768 mole), and 95% ethanol (1500 ml.) were heated until most of the solids had dissolved. Water (200 ml.) was added, and the mixture was refluxed for 2 hours. After cooling, the precipitate was filtered off, taken up in 95% ethanol (about 5 gal.)(crystallization from glacial acetic acid was unsatisfactory), boiled down (to about 2 gal.), and allowed to crystallize. A second and third crop, also of reasonably good melting point, raised the yield to 211.0 g. (0.646 mole, 42%).

4,4'-Diaminobenzil

The procedure of Gee and Harley-Mason (160) was unsatisfactory, since very little of the product dissolves in boiling, concentrated hydrochloric acid.

4,4'-Diacetamidobenzil (160) (18.00 g., 0.055 mole) was boiled (oil-bath at 135-140°) with concentrated hydrochloric acid (100 ml.; 50 ml. more added after 15 minutes) for 30 minutes, cooled, and basified with ammonium hydroxide. The precipitate was filtered off and crystallized from 95% ethanol (75 ml.)-water (250 ml.) to give 8.68 g. (0.0361 mole, 65%) of product, m.p. 159-160°.

4,4'-Bis[4"-(bis(β-chloroethyl))amino)benzylidene]diaminobenzil (CLVIII)

4,4'-Diaminobenzil (9.61 g., 0.040 mole) and p-[N,N-bis(2-chloroethyl)amino]benzaldehyde (19.69 g., 0.080 mole) were stirred at room temperature in absolute ethanol (330 ml.). After 15.5 hours, 15.60 g. of bright yellow precipitate (m.p. 145-151° put in bath at 133°) were filtered off, and the filtrate was stirred 8.5 hours more to give 2.95 g. The total was 18.55 g. (0.0266 mole, 67%). For crystallization, the product was heated very rapidly (4 minutes, with the hot-plate on "high", with stirring) to boiling in nitromethane (26.73 ml. per g.), treated with Norite, filtered, and set into the refrigerator without re-heating to dissolve any precipitate. In this way, a yield of 65% of fine, bright yellow needles was obtained. M.p. 180-181° (put in bath at

163°). Longer heating rapidly decreased the yield and produced a darker product. The analytical sample was crystallized once from nitromethane, m.p. 186-187° (put in bath at 163°).

Anal. Calcd. for $C_{36}H_{34}Cl_4N_4O_2$:

C, 62.08%; H, 4.92%; N, 8.04%.

Found: C, 61.88%; H, 4.76%; N, 7.99%.

BIBLIOGRAPHY

1. Cancer Chemotherapy Reports, 16, 1-610 (1962).

2. A. Gilman and F. S. Phillips, Science, 103, 409 (1946).

3. O. V. St. Whitelock (Editor), Ann. N. Y. Acad. Sci., 68, 657-11266 (1958).

4. S. M. Farhat, N. S. Amer, D. S. Weeks, and M. M. Musselman, A. M. A. Arch. Surg., 76, 749 (1958); C.A., 52, 12213 (1958).

5. E. G. Trams, M. V. Nadkarni, and P. K. Smith, Cancer Research, 21, 560, 567 (1961).

6. W. Jacobson, "Biological Aspects of Chemotherapy with Folic Acid Antagonists," in Biological Approaches to Cancer Chemotherapy, ed. R. J. C. Harris, Academic Press, Inc., New York, N. Y., 1961.

7. D. W. Wooley and J. M. Stewart, Biochem. Pharmacol., 8, 72 (1961).

8. A. D. Welch, Cancer Research, 21, 1475 (1961).

9. B. R. Baker, W. W. Lee, W. A. Skinner, A. P. Martinez, and E. Tong, J. Med. Pharm. Chem., 2, 633 (1959).

10. J. F. Danielli, "General Cell Theory and Drug Design," in Biological Approaches to Cancer Chemotherapy, ed. R. J. C. Harris, Academic Press, Inc., New York, N. Y., 1961.

11. S. Weinhouse, "Enzymatic Activities and Tumor Progression," in Amino Acids, Proteins, and Cancer Biochemistry, ed. J. T. Edsall, Academic Press, Inc., New York, N. Y., 1960.

12. R. A. Peters, "Lethal Synthesis," in Biological Approaches to Cancer Chemotherapy, ed. R. J. C. Harris, Academic Press, Inc., New York, N. Y., 1961.

13. J. A. Jacquez, C. C. Stock, and R. K. Barclay, Cancer, 6, 828 (1953).

14. R. T. Williams, "Detoxication Mechanisms and the Design of Drugs," in Biological Approaches to Cancer Chemotherapy, ed. R. J. C. Harris, Academic Press, Inc., New York, N. Y., 1961.

15. E. Boger and O. M. Friedman, J. Am. Chem. Soc., 80, 2583 (1958).

16. O. M. Friedman and R. Chatterji, J. Am. Chem. Soc., 81, 3750 (1959).

17. A. P. Martinez, W. A. Skinner, W. W. Lee, L. Goodman, and B. R. Baker, J. Am. Chem. Soc., 82, 6050 (1960).

18. W. C. J. Ross, Biochem. Pharmacol., 8, 235 (1961).

19. R. C. Elderfield, I. S. Covey, J. B. Geiduschek, W. L. Meyer, A. B. Ross, and J. H. Ross, J. Org. Chem., 23 1749 (1958).

20. R. Hirt and R. Buchtold, Cancer Chemotherapy Reports, 18, 5 (1962).

21. F. Bergel, "Chemistry of Enzymes in Cancer," Charles C. Thomas, Springfield, Illinois, (1961).

22. A. C. Griffin, "Effect of Cancer Chemotherapeutic Agents Upon Cellular Metabolism and Composition," in Cancer Chemotherapy, ed. I. N. Kugelmass, C. C. Thomas, Springfield, Illinois, (1961).

23. E. Wilson and M. Tishler, J. Am. Chem. Soc., 73, 3635 (1951).

24. G. V. Rao and C. C. Price, J. Org. Chem., 27, 205 (1962).

25. F. Bergel, J. L. Everett, J. J. Roberts, and W. C. J. Ross, J. Chem. Soc., 1955, 3838.

26. R. Jones, Jr., C. C. Price, and A. K. Sen, J. Org. Chem., 22, 783 (1957).

27. Y. Kuwada, Chem. Pharm. Bull. (Tokyo), 8, 77 (1960).

28. F. D. Popp and W. Kirsh, J. Org. Chem., 26, 3858 (1961).

29. F. D. Popp, J. Med. Pharm. Chem., 5, 627 (1962).

30. F. D. Popp, J. Org. Chem., 26, 3091 (1961).

31. Von W. Schulze, J. prakt. Chem., 17, 24 (1962).

32. Von W. Schulze, J. prakt. Chem., 14, 17 (1961).

33. W. C. J. Ross, G. P. Warwick, and in part, J. J. Roberts, J. Chem. Soc., 1955, 3110.

34. M. Ishidate, Y. Sakurai, and Y. Kuwada, Chem. Pharm. Bull. (Tokyo), 8, 543 (1960).

35. Von W. Schulze and G. Letsch, J. prakt. Chem., 14, 11 (1961).

36. H. Hellmann and G. Opitz, "α-Aminoalkylierung," Verlag Chemie, G.m.b.H., Weinheim, Germany, 1960.

37. B. Reichert, "Die Mannich-Reaktion," Springer-Verlag, Berlin, Germany, 1959.

38. R. C. Elderfield and J. R. Wood, J. Org. Chem., 26, 3042 (1961)..

39. W. R. Vaughan, M. S. Habib, R. S. McElhinney, N. Takahashi, and J. A. Waters, J. Org. Chem., 26, 2392 (1961).

40. G. R. Pettit and D. S. Alkalay, J. Org. Chem., 25, 1363 (1960).

41. Y. Tsuchiki, H. Wada, and Y. Suzuki, Japan. 918 (Feb. 18, 1960); C.A., 54, 19539 (1960).

42. W. L. Nylan and H. Busch, Chem. Eng. News, 46 (May 5, 1958).

43. O. M. Friedman and A. M. Seligman, J. Am. Chem. Soc., 70, 3082 (1948).

44. M. H. Benn, A. M. Creighton, L. N. Owen, and G. R. White, J. Chem. Soc., 1961, 2365.

45. G. A. Usbeck, J. W. Jones, and R. K. Robins, J. Am. Chem. Soc., 83, 1113 (1961).

46. K. A. Kornev and K. K. Khomenkova, Ukrain. Khim. Zhur., 25, 484 (1959); C.A., 54, 9900 (1960).

47. I. Ya. Postovskii, E. I. Grinblat, L. F. Trefilova, J. Gen. Chem. U.S.S.R. (Eng. Transl.), 31, 363 (1961).

48. M. Ishidate, Y. Sakurai, and Y. Kuwada, Chem. Pharm. Bull. (Tokyo), 8, 732 (1960).

49. D. Fles and A. Markovac-Prpic, Arhiv. kem., 27, 211 (1955); C.A., 50, 11951 (1956).

50. S. Peterson and W. Gauss, Ger. 939,151 (Feb. 16, 1956; Cl. 12 p, 5); C.A., 53, 8096 (1959); Chem. Zentr., 1957, 2063.

51. J. Degutis and D. Sukeliene, Zhur. Vsesoyuz. Khim. Obshchestva im. D. I. Mendeleeva, 6, 583 (1961); C.A., 56, 5908 (1962).

52. E. Plotz and E. Weinhardt, Ger. 858,700 (Dec. 8, 1952; Cl. 12 p); C.A., 52, 5456 (1958).

53. G. Sunagawa, K. Murayama, and N. Yoshida, Yakugaku Zasshi, 77, 1173 (1957); C.A., 52, 6303 (1958).

54. H. Bestian, G. Ehlers, E. Plotz, and G. Steinbrunn, Ger. 849,407 (Sept. 15, 1952; Cl. 12 p, 5); C.A., 52, 20195 (1958); Chem. Zentr., 1954, 1353.

55. K. Vierling, Ger. 935,545 (Nov. 24, 1955; Cl. 12 p, 5); C.A., 52, 20195 (1958); Chem. Zentr., 1956, 7661.

56. H. Bestian, Ann., 566, 210 (1950).

57. T. S. Safonova and S. I. Sergievskaya, J. Gen. Chem. U.S.S.R. (Eng. Transl.), 30, 2414 (1960).

58. M. Izumi, Chem. Pharm. Bull. (Tokyo), 2, 275 (1954).

59. M. Ishidate, Y. Sakurai, and M. Izumi, J. Am. Pharm. Assoc., 44, 132 (1955).

60. M. Izumi, Japan. 3767 (May 23, 1956); C.A., 51, 14792 (1957).

61. H. Kondo, H. Kataoka, and T. Dodo, Itsuu Kenkyusho Nempo, 11, 53 (1960); C.A., 55, 17619 (1961).

62. J. DeGraw and L. Goodman, J. Org. Chem., 27, 1395 (1962).

63. J. DeGraw and L. Goodman, J. Org. Chem., 27, 1728 (1962).

64. R. C. Elderfield and J. R. Wood, J. Org. Chem., 27, 2463 (1962).

65. A. M. Rutenburg, L. Persky, O. M. Friedman, and A. M. Seligman, J. Pharmacol. Exptl. Therap., 111, 483 (1954); C.A., 48, 13960 (1954).

66. S. N. Nagaraja and S. W. Sunthankar, J. Sci. Ind. Res. (India), 20D, 378 (1961); Index Chemicus, 4, entry 14665 (1962).

67. M. Ishidate, Y. Sakurai, and M. Torigoe, Chem. Pharm. Bull. (Tokyo), 9, 485 (1961).

68. G. R. Pettit and J. A. Settepani, J. Org. Chem., 27, 1714 (1962).

69. Von W. Schulze and G. Letsch, J. prakt. Chem., 16, 182 (1962).

70. L. Larionov, Cancer Chemotherapy Reports, 12, 205 (1961).

71. F. R. White, Cancer Chemotherapy Reports, 14, 169 (1961).

72. J. Kennedy and A. Theologides, Cancer Chemotherapy Reports, 11, 77 (1961).

73. H.-L. Chou, H.-C. Lin, and C.-Y. Yuan, K'o Hsueh T'ung Pao, 14, 457 (1959); C.A., 54, 7716 (1960).

74. R. B. Ross and P. E. Swartzentruber, Literature Survey of Nitrogen Mustards, National Institutes of Health, Bethesda, Md., 1959.

75. A. Benitez, L. O. Ross, L. Goodman, and B. R. Baker, J. Am. Chem. Soc., 82, 4585 (1960).

76. C. C. Price and H. H. Lin, J. Org. Chem., 26, 266 (1961).

77. K. A. Kornev and K. K. Khomenkova, Ukrain. Khim. Zhur., 26, 347 (1960); C.A., 55, 3599 (1961); Index Chemicus, 2, entry 5394 (1961).

78. J. Farkas and F. Sorm, Collection Czeckoslav. Chem. Communs., 26, 893 (1961).

79. L. O. Ross, W. W. Lee, M. G. M. Schelstraete, L. Goodman, and B. R. Baker, J. Org. Chem., 26, 3021 (1961).

80. F. Sorm and J. Vesely, Experientia, 17, 355 (1961).

81. D. A. Lyttle and H. G. Petering, U.S. 1,983,727 (May 9, 1961); C.A., 55, 19966 (1961).

82. H. Segal and C. G. Skinner, J. Org. Chem., 27, 199 (1962).

83. J. H. Lister and G. M. Timmis, J. Chem. Soc., 1960, 1113.

84. J. I. DeGraw, L. O. Ross, L. Goodman, and B. R. Baker, J. Org. Chem., 26, 1933 (1961).

85. R. C. Elderfield and R. N. Prasad, J. Org. Chem., 24, 1410 (1959).

86. Personal communication from Dr. R. C. Elderfield.

87. S. Chu, J. E. Harris, and H. G. Mautner, J. Org. Chem., 25, 1759 (1958).

88. G. DiPaco and C. S. Tauro, Ann. chim. (Rome), 47, 698 (1957); C.A., 52, 1179 (1958).

89. G. Huber, Angew. Chem., 68, 706 (1956).

90. D. A. Lyttle and H. G. Petering, U.S. 2,957,875 (Oct. 25, 1960); C.A., 55, 8445 (1961).

91. S. H. Burstein and H. J. Ringold, Can. J. Chem., 40, 561 (1962).

92. T. P. Johnston, A. L. Fikes, and J. A. Montgomery, J. Org. Chem., 27, 973 (1962).

93. H. H. Lin and C. C. Price, J. Org. Chem., 26, 264 (1960).

94. H. C. Koppel, R. H. Springer, R. K. Robins, F. H. Schneider, and C. C. Cheng, J. Org. Chem., 27, 2173 (1962).

95. R. G. Gushkov, E. S. Golouchinskaya, and O. Yu. Magidson, J. Gen. Chem. U.S.S.R. (Eng. Transl.), 29, 3700 (1959).

96. W. S. Gump and E. J. Nikawitz, J. Org. Chem., 24, 712 (1959).

97. O. F. Ginzburg, B. A. Porai-Koshits, M. I. Krylova, and K. Yu. Mar'yanovskaya, Khim. Nauka i Prom., 4, 548 (1959); C.A., 54, 8794 (1960).

98. O. F. Ginzburg, B. A. Porai-Koshits, and K. Yu. Mar'yanovskaya, J. Gen. Chem. U.S.S.R. (Eng. Transl.), 30, 591 (1960).

99. Z. V. Pushkareva and L. V. Alekseeva, Zh. Obshch. Khim., 32, 1058 (1962).

100. L. V. Alekseeva and Z. V. Pushkareva, J. Gen. Chem. U.S.S.R. (Eng. Transl.), 31, 2394 (1961).

101. S.-C. J. Fu, J. Med. Pharm. Chem., 5, 33 (1962).

102. L. V. Alekseeva and Z. V. Pushkareva, J. Gen. Chem. U.S.S.R. (Eng. Transl.), 31, 2719 (1961).

103. J. C. Sheehan, D. W. Chapman, and R. W. Roth, J. Am. Chem. Soc., 74, 3822 (1952).

104. I. Schumann and R. A. Boissonnas, Helv. Chim. Acta, 35, 2235 (1952).

105. M. Sela and E. Katchalski, Advances in Protein Chemistry, XIV, 391 (1959).

106. L. Delmonte and T. H. Jukes, Pharmacological Reviews, 14, 108 (1962).

107. W. E. Knox, in Physiological Adaption, American Physiological Society, Washington, D. C., 1958.

108. J. Claudatus and S. Ginori, Science, 125, 394 (1957).

109. S. Wood, Jr., R. S. Rivlin, and W. E. Knox, Cancer Research, 16, 1053 (1956).

110. S. Udenfriend, H. Weissbach, and A. Sjoerdsma, Science, 123, 669 (1956).

111. P. Barjon, A. C. de Paulet, and O. Flandre, Pathol. Biol. Semaine Hop., 10, 69,75 (1962); C.A., 56, 14862, 14863 (1962).

112. J. W. Cole and L. M. Matthews, A.M.A. Arch. Surg., 76, 912 (1958); C.A., 52, 13961 (1958).

113. W. C. J. Ross and J. G. Wilson, J. Chem. Soc., 1959, 3616.

114. G. R. Pettit, M. F. Baumann, and K. N. Rangammal, J. Med. Pharm. Chem., 5, 800 (1962).

115. R. L. Hinman, J. Am. Chem. Soc., 78, 2463 (1956).

116. E. E. Royals, "Advanced Organic Chemistry," Prentice Hall, Inc., Englewood Cliffs, N. J., 1954; p. 649.

117. F. D. Popp, J. Org. Chem., 26, 1566 (1961).

118. Chem. Eng. News, 47 (March 19, 1962).

119. G. R. Pettit and J. A. Settepani, J. Med. Pharm. Chem., 5, 296 (1962).

120. H. V. Dobeneck, W. Lehnerer, and G. Maresch, Hoppe-Seyler's Z. Physiol. Chem., 304, 26 (1956); C.A., 51, 17883 (1957).

121. H. Hellmann and G. Opitz, "α-Aminoalkylierung," Verlag Chemie, G.m.b.H., Weinheim, 1960.

122. B. Reichert, "Die Mannich-Reaktion," Springer-Verlag, Berlin, 1959.

123. Y. F. Shealy, C. A. Krauth, and J. A. Montgomery, J. Org. Chem., 27, 2150 (1962).

124. Y. F. Shealy, R. F. Struck, L. B. Holum, and J. A. Montgomery, J. Org. Chem., 26, 2396 (1961).

125. S. E. Lewis, E. D. Wills, and A. Wormall, Intern. Radiation Biol., 3, 647 (1961); C.A., 56, 3777 (1962).

126. G. Honti and Gy. Putnoky, Z. Ges. Inn. Med. u. Ihre Grenzgebiete, 16, 750 (1961); C.A., 56, 9368 (1962).

127. S. Hatem, Chimia, 13, 158 (1959); C.A., 54, 15619 (1960).

128. K. Yamana, T. Shimizu, H. Shigekane, and M. Kodama, Nagoya J. Med. Sci., 19, 193 (1957); C.A., 53, 22471 (1959).

129. M. C. Champy, Bull. acad. natl. med. (Paris), 3, 143, 288 (1959); C.A., 53, 16343 (1959).

130. R. M. S. Smellie, R. Y. Thompson, and J. N. Davidson, Biochim. et Biophys. Acta, 29, 59 (1958); C.A., 52, 18813 (1958).

131. D. A. Clarke, H. C. Reilly, and C. C. Stock, Antibiotics and Chemotherapy, 7, 653 (1957); C.A., 52, 13121 (1958).

132. G. J. Martin, "Biological Antagonism," The Blakiston Company, Inc., New York, 1951.

133. A. Albert, Quart. Rev., 6, 221 (1952).

134. A. Albert, "Some Unresolved Problems," in Chemistry and Biology of Pteridines, ed. G. E. W. Wolstenholme and M. P. Cameron, Little, Brown, and Company, Boston, 1954.

135. E. Drechsel, J. prakt. Chem., 135, 418 (1883).

136. J. C. Sheehan and V. S. Frank, J. Am. Chem. Soc., 71, 1856 (1949).

137. D. Fles and A. Markovac-Prpic, Arhiv. kem., 27, 211 (1955); C.A., 50, 11951 (1956).

138. A. T. de Mouilpied, J. Chem. Soc., 87, 438 (1905).

139. C. H. Bamford, A. Elliott, and W. E. Hanby, "Synthetic Polypeptides," Academic Press, Inc., 1956.

140. F. G. Mann, J. Chem. Soc., 1934, 461.

141. K. N. F. Shaw, A. McMillan, A. G. Gudmundson, and M. D. Armstrong, J. Org. Chem., 23, 1177 (1958).

142. A. R. Katritzky, J. Chem. Soc., 1955, 2581.

143. R. C. Elderfield, B. Fischer, and J. M. Lagowski, J. Org. Chem., 22, 1376 (1957).

144. F. P. Doyle, Mrs. W. Ferrier, D. O. Holland, M. H. Mehta, and J. J. C. Naylor, J. Chem. Soc., 1956, 2853.

145. Upjohn Co., Brit. 778,823 (July 10, 1957); C.A., 52, 1265 (1958).

146. F. Millich and E. I. Becker, J. Org. Chem., 23, 1096 (1958).

147. A. P. Terentyev, A. N. Kost, and V. A. Smit, J. Gen. Chem. U.S.S.R. (Eng. Transl.), 26, 597 (1956).

148. J. Harley-Mason, Chem. and Ind., 1951, 886.

149. L. I. Smith and A. W. Sogn, J. Am. Chem. Soc., 67, 822 (1945).

150. R. H. F. Manske and R. Robinson, J. Chem. Soc., 1927, 240.

151. J. N. Ashley and R. Robinson, J. Chem. Soc., 1928, 1376.

152. P. E. Peterson, J. P. Wolf III, and C. Nieman, J. Org. Chem., 23, 303 (1958).

153. R. Mauima and M. Kotake, Chem. Ber., 55, 3861, 3867 (1922).

154. A. Ellinger, Chem. Ber., 39, 2519 (1906).

155. S. Kakimoto and J. Nishie, Japan. J. Tuberc., 2, 334 (1954); C.A., 50, 14744 (1956).

156. R. K. Brown, R. F. Snider, and M. D. Stevenson, J. Org. Chem., 21, 261 (1956).

157. J. J. Donleavy and M. A. Kise, Org. Syn., 17, 63 (1937).

158. R. N. Castle and W. S. Seese, J. Org. Chem., 23, 1534 (1958).

159. R. G. Jones, J. Am. Chem. Soc., 71, 383 (1949).

160. H. L. Gee and J. Harley-Mason, J. Chem. Soc., 1947, 251.

161. W. S. Ide and J. S. Buck, Organic Reactions, IV, 280 (1948).

162. H. H. Hodgson and H. G. Beard, J. Chem. Soc., 1927, 21.

163. R. Preussmann, Arzneimittel-Forsch., 12, 260 (1962).