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Potential neoplasm inhibitors

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POTENTIAL NEOPLASM INHIBITORS

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by

Andrew G. Bachmann

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Dedicated to my parents,

Andrew J. and Edna R. Bachmann

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INTRODUCTION[7]

1. Cancer

"Next to cardiovascular disorders, cancer is the greatest killer in most western countries. Perhaps it has always been that way, but improved methods of diagnosis and the advancing age of the world's population during the last half century have raised cancer, primarily a disease of older age groups, to the second place on the blacklist of vital statistics. Even though cancer was recognized as a disease by the Egyptians over 3500 years ago, the cause or causes of spontaneous cancer are unknown, and so is any cure for it. Until reliable diagnostic tests for the early detection of the various malignancies in man are developed, one may expect a further increase in the mortality from the different diseases which are called cancer. At present only one-third of all cancer patients survive 5 years after diagnosis has been made and some sort of therapy has been instituted. This is due to the ability of neoplastic diseases to invade other tissues (to metastasize) if a malignant cell floats away in the body fluids and locates in a distant place of the organism. The role of chemotherapy becomes particularly understandable in such disseminated cancers which can no longer be attacked by localized surgery or irradiation.

Although most types of cancer have been and are on the increase, the incidence of cancer of the lung and pharynx has risen in an unusually steep slope. This has been attributed variously to similar increases in cigaret smoking, exhaust gases from combustion engines,

asphalt roads which might slowly release carcinogens, industrial smog, etc. Although some of the pertinent comparative statistics sound convincing, no evidence exists that any of these conditions or activities are causative in producing cancer in humans. Of other tumors, cancer of the stomach has declined slowly over the last 30 years.

Malignant tumors may be induced in animals and man in many different ways. Repeated and constant physical irritation (light, heat) can produce cancer, and so can many chemicals. Of these chemical carcinogens or cancerogens, polycyclic aromatic hydrocarbons and their nitrogen-heterocyclic isosteres have been studied most intensively. Certain azo dyes, aromatic amines, carbamates, and other structural types can also induce malignant, transplantable tumors. A few compounds in these series (urethan, 2-aminochrysene, etc.) also exhibit tumor-inhibitory action, but their showing has not been impressive."

2. History of Chemotherapy

"The use of folk remedies in the treatment of cancer and other tumors is recorded early in the history medicine. For example, an ancient Egyptian remedy was to breath the vapors of boiling wine. For obvious reasons, this was a very popular treatment. However, the development of the drug treatment of cancer has not paralleled that of medicine in general nor the drug treatment of other diseases in particular. Only in the past few years has cancer chemotherapy been allotted any appreciable portion of the resources of medical and

biological research groups. As in other fields of medicinal chemistry, the improvement of the understanding of the biochemical background of drug action has made it possible to pinpoint important structural areas in which active antineoplastic drugs might be found. Nevertheless, the urgency of cutting down the increasing death rate from cancer does not permit one to concentrate on logical approaches of cancer chemotherapy only. As long as the origin of spontaneous cancer remains totally unknown and the question whether cancer is one single disease or a group of symptoms of many fundamentally different diseases has not been settled, even the slightest empirically obtained lead cannot be disregarded.

The discovery of the antileukemic activity of the nitrogen mustards was a turning point in the approach to cancer chemotherapy. During World War II, a vessel carrying mustard gas (bis-2-chloroethyl sulfide) was bombed in an Italian harbor, and the vesicant material was spread over the waves. Combat personnel thrown into the water by the explosion were rescued after some time, and treated for the effects of the war gas. Many of these patients developed leucopenia, a blood-dyscrasia manifested by a dangerous reduction of the leucocytes. Since a decrease of white cells would be an indication of improvement in certain leukemias where white cells are overproduced by the neoplastic bone marrow, bischloroethyl sulfide was tested in leukemia patients. The high toxicity of the material was prohibitive, but led by structural analogy, to a study of polyvalent nitrogen mustards mostly bischloroethylamines, in blood cancer. Several hundreds of these substances have been tested as well as numerous compounds presumed to have similar activity. Although none of these

compounds constituted a cure for any type of human neoplasm, their tumor retarding effects raised the hope that curative compounds might be found in the long run.

A new impetus was given to cancer chemotherapy in 1948 when Farber observed that certain analogs of pteroyl-glutamic (folic) acid had antineoplastic activity. This discovery was triggered by the knowledge that folic acid promotes the production of blood corpuscles and the idea that structurally related compounds might act as antagonists, in conditions such as leukemia where retardation of the production of certain types of blood cells is desirable. Work on potential antagonists in other series of compounds involved in cancer growth led to the discovery of anti-neoplastic activity in certain steroids, purine and pyrimidine derivatives, as well as isolated instances in other types of compounds.

Purpose and Scope of the Research

The historical portion of this paper is designed to show the value of mustard compounds in the treatment of cancer and the methods used to take advantage of various exploitable variants between normal tissue and cancerous tissue.

The experimental section is an effort to evaluate and take advantage of the variant of pH.

HISTORY

Biological Activity of the Alkylating Agents[8,33]

A compound which can effect the replacement of a hydrogen atom in a molecule by an alkyl group or whose radical may add to a molecule containing an atom in a lower valency state is called an alkylating agent. X-H represents any

$$X\text{-}H \longrightarrow X\text{-}R$$
$$NR_3 \longrightarrow R'\text{-}N^{+}R_3$$

molecule with an active or replaceable hydrogen and R- represents an alkyl group. In the case of biological alkylating agents R- is normally restricted to saturated compounds.

Alkylating agents fall into two large classes according to their mechanisms of reaction.

Class I: S_N1 mechanism

This type of mechanism is dependent upon the solvent for separation of the ions. It is of limited use as a biological

$$R\text{-}Y \xrightarrow{(slow)} R^{+}(solvent) + Y^{-}(solvent)$$

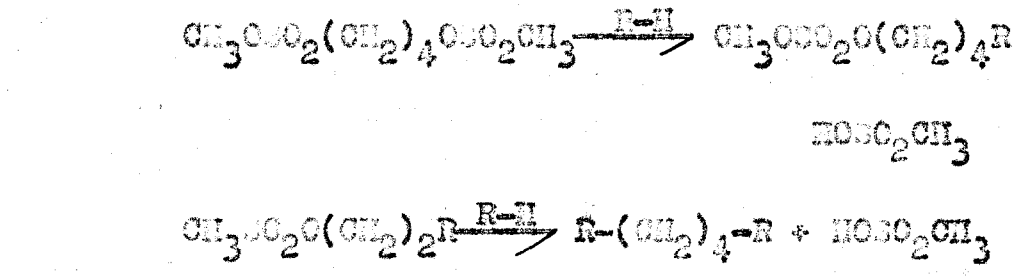
$$R^{+}(solvent) + X^{-}(solvent) \xrightarrow{(fast)} RX + solvent$$

alkylating agent because the carbonium ion (R^{+}) is much more likely to react with the stabilizing solvent sheath than with biological systems.

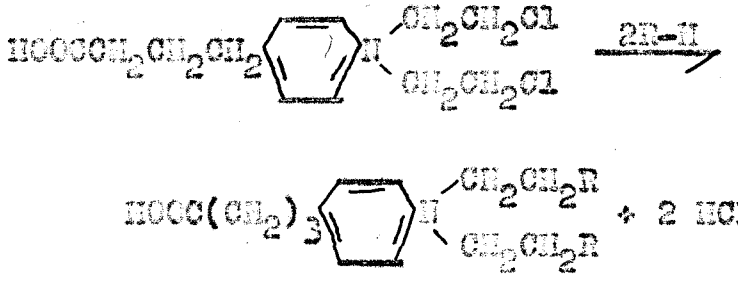
The alkanesulfonyl sulfonyl esters and 2-chloroethyl aryl amines make up most of this class.

Examples:

a) 1,4-(dimethanesulfonoxy)butane (Myleran)



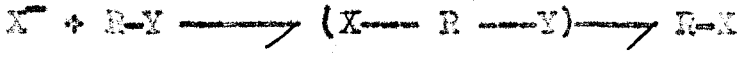
b) p- di-(2-chloroethyl)amino phenylbutyric acid (Chlorambucil)



It will be noted, however, that *in vivo* these compounds *may* exhibit an S_N2 mechanism.

Class II: S_N2 mechanism

In this mechanism complete separation of R and Y does not occur but a transition state in which R is loosely combined with both X and Y is postulated.



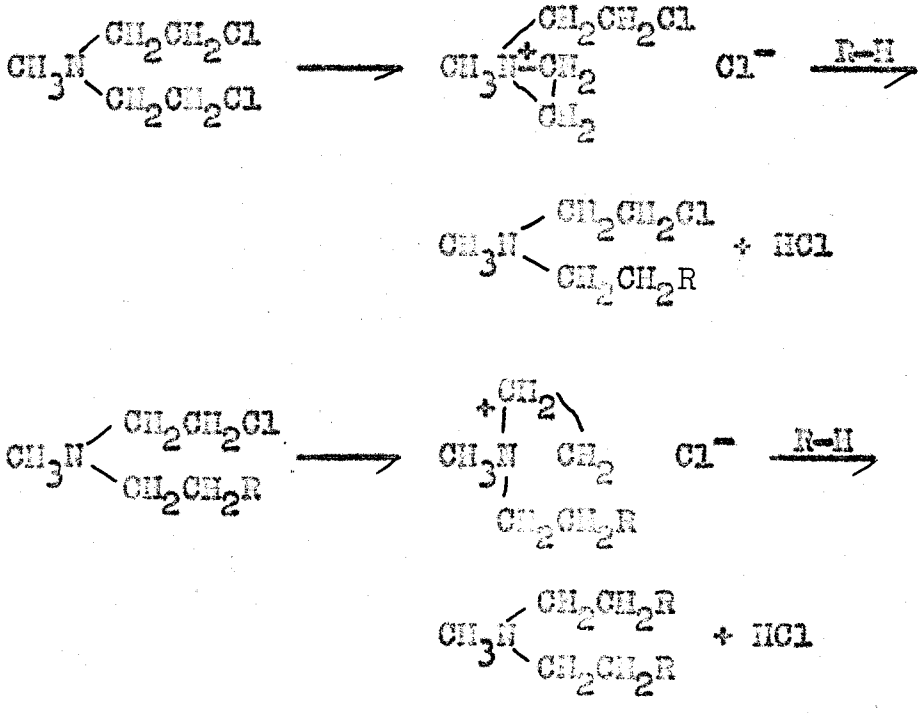
The rate of an S_N2 mechanism, in contrast with the S_N1, is more dependent upon the concentration of the displacing group X and upon its affinity for the nucleophilic R^+.

Since there is only consequential reaction with solvent, this class is the most desirable for use as biological alkylating agents.

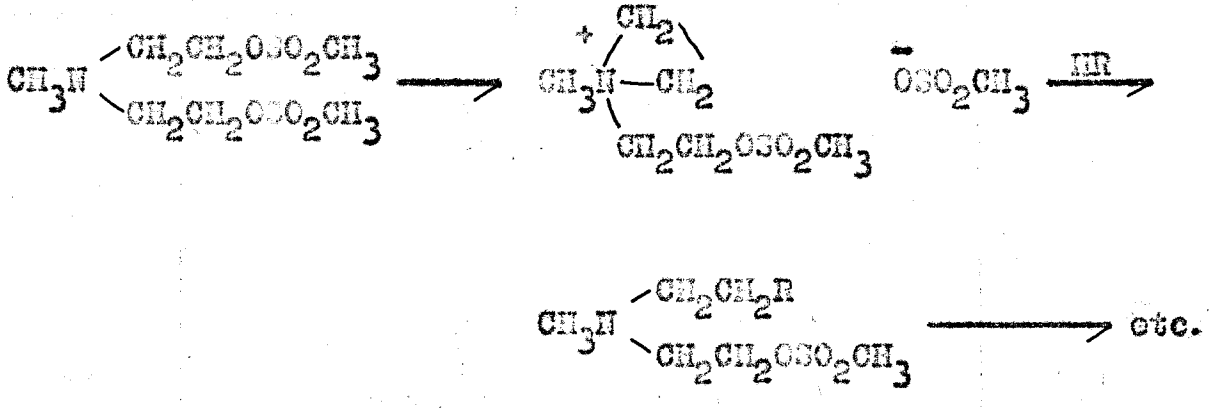
In this class are the sulfur and nitrogen mustards and their methane sulfonyl ester analogues. In most of these compounds alkylation is preceded by the formation of a cyclic intermediate which precludes the design of compounds with a permanent positive charge on the nitrogen atom.[52,33]

Examples:

a) N-methyl-N,N-bis(2 chloroethyl) amine (HN2)



b) The same mechanism exists for the methane sulfonyl analogues.



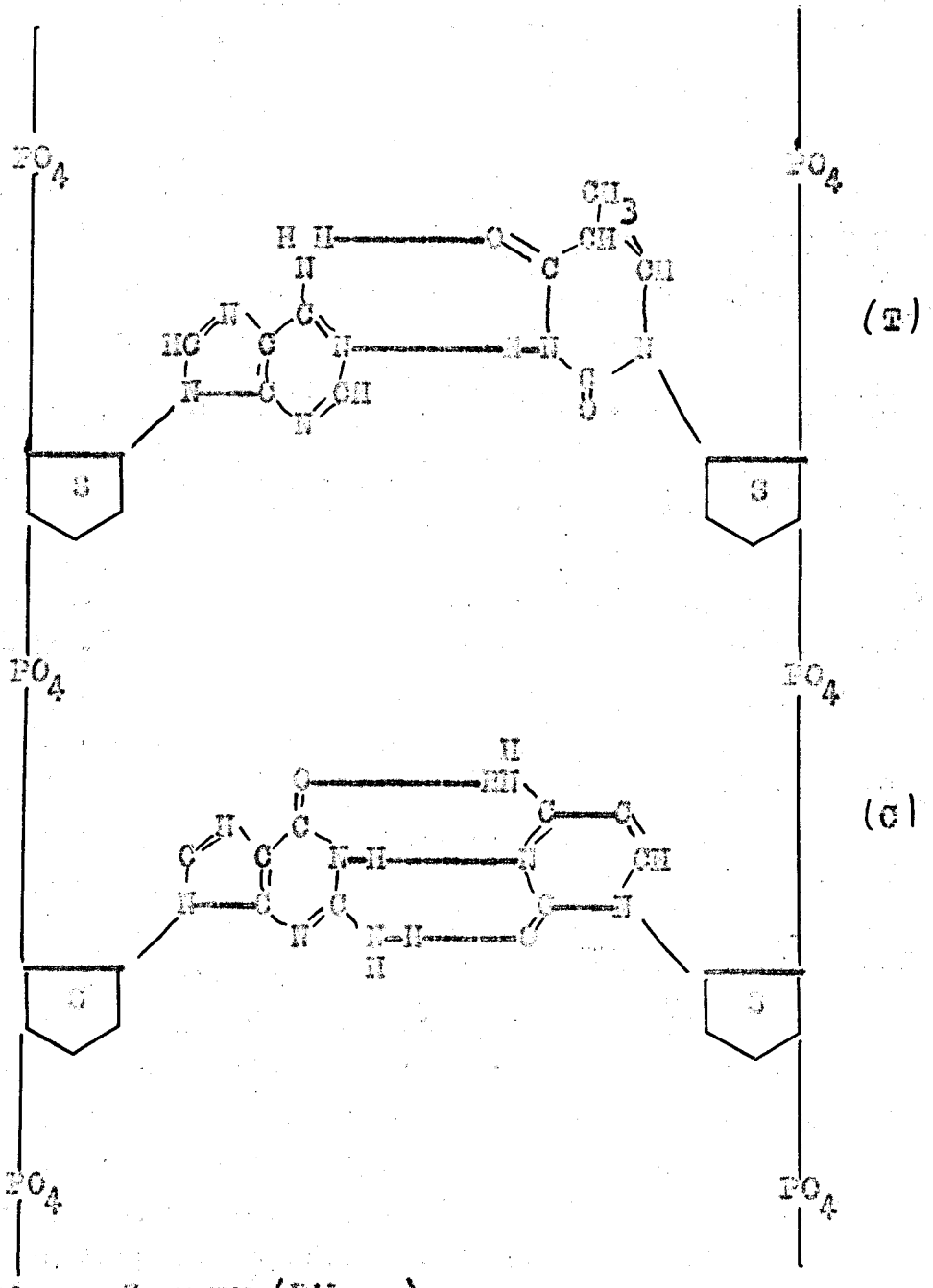
In a living cell it is evident that these alkylating agents may react with a multitude of molecules containing active hydrogens; water, alcohols, thiols, acetals, ketals, acids, amines, etc. Since so many constituents of a cell are liable to attack, to be lethal, the alkylating agent must enter the nucleus and react with the genetic material, DNA, deactivating it and thereby causing a fatal mutation of the cell.[34]

The "ladders" of the DNA molecule are held together by hydrogen bonding. (see page 5)

Acids and alkalies cause irreversible reductions in the viscosity of DNA solutions indicating a rupture of the hydrogen bonds between amino and hydroxyl groups of adjacent bases. Even in low concentrations, alkylating agents also reduce viscosity of DNA solutions. In alkylating the free amino groups of the purine or pyrimidine bases, the hydrogen bonding maintaining the stiffness of the molecule is destroyed.[22]

Myleran does not kill cells outright but simply interrupts mitosis.[47] It may be that the Myleran type of compound adds across the DNA molecule, between amino groups,

thereby destroying the molecules ability to divide and reproduce itself.



A=Adenine S=Sugar (Ribose)
T=Thymine
G=Guanine
C=Cytosine

EXPLOITABLE VARIANTS

The problem in chemotherapy is twofold. One needs to have an agent which is especially toxic toward dividing cells and in addition one needs an agent which acts specifically on the type of tissue in which the particular neoplasm arises. Alkylating agents satisfy the first requirement in that they are especially effective against tissues with a high mitotic index, but they also are general toxins. It is therefore desirable to introduce some specificity into chemotherapeutic agents.

I) Mechanistic:[35]

Compounds which react by the S_N2 Mechanism will be more involved in regions where there is a high concentration of nucleophilic groups, for example adjacent to nucleic acid chains and in thiol rich areas. It will be an advantage for compounds which react by the S_N2 mechanism to have low intrinsic reactivity since this will enable them to survive long enough to reach effective areas.

II) Methanesulphonates:[36]

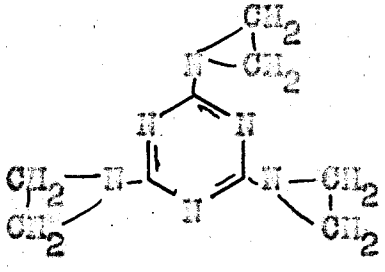
In the series $CH_3SO_2O(CH_2)_nOSO_2CH_3$, the chemical reactivity is low in compounds(n=2and3) due to the mutual deactivating effect of the methanesulphony groups. From n=4 upwards the chemical reactivity is higher and more uniform.

The introduction of alpha methyl groups into the above structure results in enhanced reactivity towards water as does the introduction of beta hydroxyl groups.

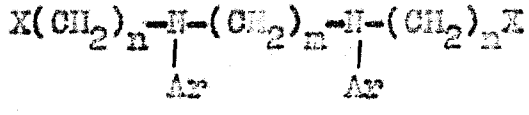
III) Functionality:[37]

Mutagenic effects are shown by many monofunctional alkylating agents, but with a few exceptions tumor inhibitory activity is only exhibited by compounds containing more than one reactive group in the molecule. For example, TMM (I) is about 25 times as active as the monofunctional derivative.

(I)



Compounds of the type



seem to be most active where m is no higher than 3 and n=2 only. (Hon and Roberts-1950)[38]

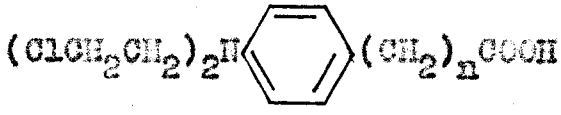
IV) Solubility:[39]

Relatively insoluble drugs are not likely to be very effective since to be carried by body fluids, a substance must, to some extent, be soluble in water.

Daniolli (1954) has suggested that, as adipose tissue is able to extract colloidal fat from the blood, tumors of fat cells should be selectively attacked by mustards with high lipoid solubility.

However, as lipoid solubility increases in HN2 types of drugs, effectiveness against leukemia decreases. Ross (1960)

has shown that compounds of the type



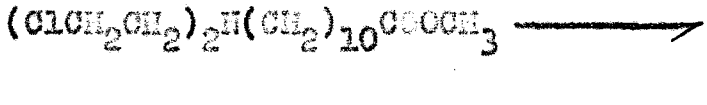
are most effective when n=3. This is the standard anti-leukemia drug, chlorambucil. The introduction of carboxyl groups also introduces ionic character, since the pKa of the acids in aqueous media is between 5 and 6.[40]

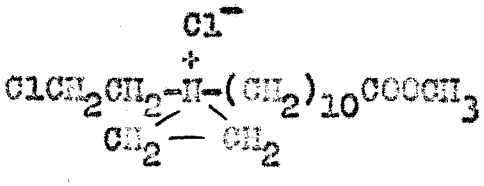
The sodium salt of chlorambucil has been used extensively for intravenous administration. In this form chlorambucil seems to be particularly rapidly removed from the blood stream. This rapid absorption may be connected with the surface active properties of the drug.[41]

The increase in water solubility has led to enhanced antitumor activity. The sodium salt of chlorambucil is about twice as reactive chemically as the aniline mustard but some twenty times as effective as a tumor inhibitor.[42]

Compounds of the type

and





are active anionic surface agents.

In compounds of the Myleran type activity seems to be highest when the ratio of water solubility to other solubil-

ty approaches 5.5 (Timmis, 1958). This would seem to be directly related to the dependence of solvent on the S_N1 mechanism. In compounds even more soluble, for example 1,6-dimethanesulphonylmannitol, the action on the blood elements was lost, but the compound is an effective inhibitor of solid tumors.[43]

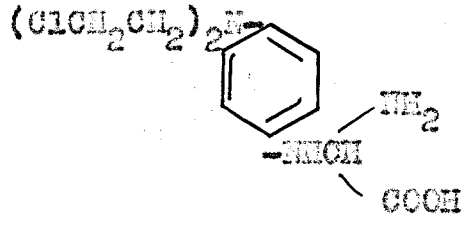
$$CH_3SO_2O(CHOH)_4SO_2CH_3$$

In general terms, one would expect that an increase in hydrophilic character in a compound which reacts by an S_N1 mechanism would enhance its effectiveness by increasing the possibility of achieving a high alkylating potential within the cell.

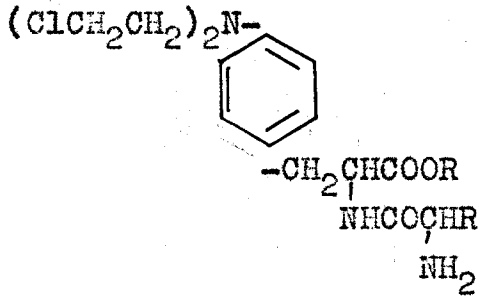
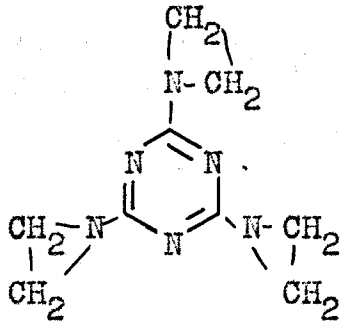
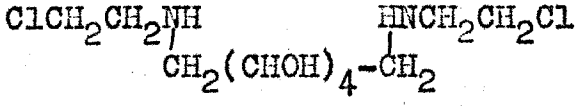
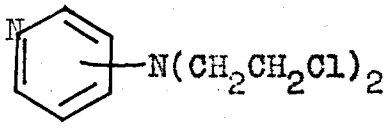
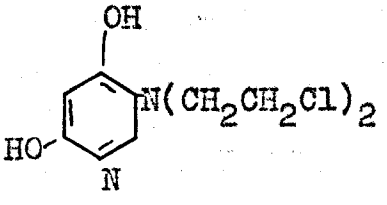
V) Compounds capable of active transport:[44]

Since, to be effective, the alkylating agents need to permeate the cell membrane and probably also the nuclear membrane, any features which assist this process should enhance activity.

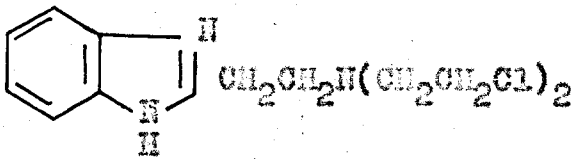
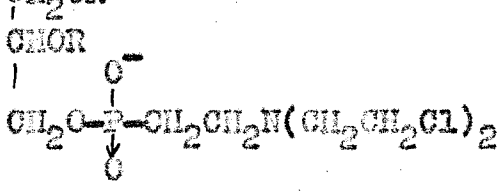
Following is a partial list of compounds which are effective because they are capable of active transport.

Transport	Formula	Trade name and reference
Amino acids	$(ClCH_2CH_2)_2NCH_2COOH$	Izumi (1954)
		(Melphan) Dorgol and Stock (1953)

(con't)

Transport	Formula	Trade name and reference
Peptides		Larinov and Sofina (1957)
Melamines		(TEM) Ross (1950)
Sugar-like molecules		(Manomustine) Vargha (1957-1958)
Pyridines		
Pyrimidines		(Dopan) Russian

(con't)

Transport	Formula	Trade name and reference
Benzimidazoles		Kirshberg, Gollhorn, and Gump (1958)
Lecithin like Phospholipids		Ross (1962)

R=$C_{17}H_{35}CO$

No outstanding activities of steriod mustards have been observed.

VII) Influence of pH on Reactivity:

"In Warburgs studies on tumors, the observation was made that, whereas both normal and tumor tissues produced lactic acid from glucose or glycogen in the absence of oxygen, practically only cancer tissue showed the ability to produce lactic acid from glucose in the presence of oxygen.

The respiration rates of cancer and of normal tissues appeared to be very nearly of the same order of magnitude, and to account for the high aerobic glycolysis in tumors Warburg postulated that the respiratory mechanism in such tissues was damaged as a result of the neoplastic transformation. High anaerobic glycolysis appeared to be a general property of growing or multiplying tissues, since it was found in embryo tissue and tactic, b[illegible] in these normal

tissues glycolysis was largely abolished by the presence of oxygen. In most tumors, the high anaerobic glycolysis was relatively little reduced when oxygen was admitted to the system.

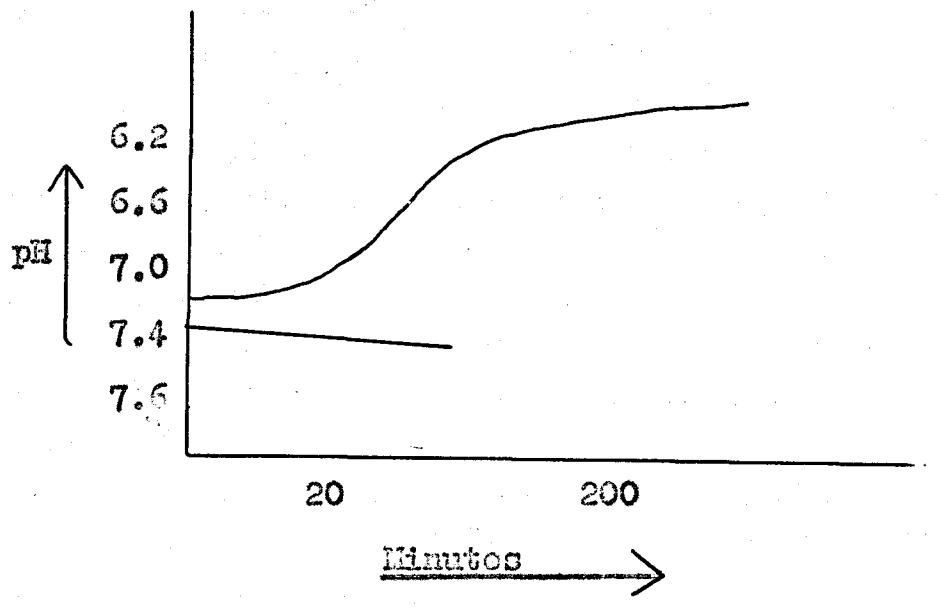
That lactic acid is actually produced from tumors was shown by several investigators who observed that the venous blood leaving a tumor contained less glucose and more lactate than did venous blood in a comparable tumor-free site in the same animal.

Since lactic acid is a relatively strong organic acid, its accumulation within the tumor should produce a lowering of the pH, provided that the rate of glycolysis exceeds both the diffusion of buffers from the arterial circulation into the cells and provided that the rate of supply of the glucose to the cells is sufficiently high.

By inserting electrodes directly into the tumor mass of the living animal, Voegtlin et al demonstrated that the administration of glucose, whether subcutaneous or intraperitoneal, was followed very quickly by a drop in pH of 0.4 to 0.6 unit. The pH of the tumor may drop from 6.9 to 6.3 without the occurrence of any pulmonary respiratory symptoms indicative of systemic acidosis, thus indicating that the increased acid production is largely confined to the tumor and that the excess lactate carried off in the venous circulation is quickly metabolized by the normal tissues.

Kahler and Robertson compared the hydrogen ion concentration of the liver and of a transplanted hepatoma in fasted rats. The pH of the liver was 7.4, that of the tumor 7.0. When the rats were supplied with excess glucose, the pH of the liver remained unaltered whereas that of the hepatoma dropped to 6.4.(Figure I).

Figure I

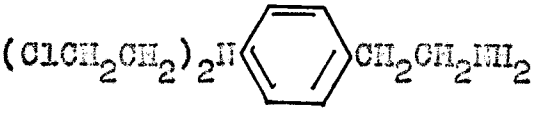


pH of Hoptoma 31 and normal liver in rat

Coris found that the lactic acid content of the mouse mammary carcinoma was 0.034%; after glucose injection the content of this acid rose to 0.137%."[19]

Stephens et al. (1950) demonstrated a selective deposition of sulphapyrazine in tumors of glucose treated rats bearing Walker rat carcinoma.[45]

Following a suggestion by Crawford (1960), that basic compounds with suitable pKa values might concentrate in tumors, some preliminary experiments were made at the Chester Beatty Research Institute with the aromatic mustard:



Some enhancement of the antitumor activity of this compound was observed when glucose was administered to a Walker

tumor-bearing rat just prior to injection of the drug.[45]

Sulphydryl compounds are readily alkylated. If a pH difference of one unit can be induced between normal and cancer cells and a sulfhydryl compound such as cysteamine is introduced in the organism, then the concentration of the more reactive sulfhydryl ion will be considerably greater in normal cells. A thiol of pKa 8 will be approximately 50% ionized at pH 7 and 1% at pH 6. This means that the mustard will be more effectively deactivated in normal cells and it should therefore act preferentially on the more acid malignant tissue.[46]

TEM has a very high value of the coefficient for the rate of acid catalysed reaction (Ross, 1950) and is an example of drugs used in this way.

Evidence for the Association of Indole With Tumor Cells

Indole has been tested for its anti-cancer properties and found lacking.[11] However, Johnston *et. al.* found that lipotropic materials such as amytol, phentothal, Br_3CCH_2OH, chlorotone and indole, which greatly inhibit brain respiration, also markedly inhibit the respiration of Erlich Ascites tumor cells. These compounds inhibit the intake of glycine into these cells under anaerobic conditions in the presence of glucose, the extent of inhibition being greater than that of anaerobic glycolysis. It is suggested that respiratory mechanisms and the transport system of the whole cell depend for their activities on the lipid components of the cell membrane structures with which these lipotropic agents become associated, thereby decreasing these activites.[23]

Biological Activity of Indoles and Pyrroles

Indole, when injected into the mouse, is adsorbed to a large degree by the intestines and is then released at an exponential rate into the body. It is also detoxified by the liver. This detoxification is accelerated by sodium santonate and vitamin C.[26]

Indole was studied as part of the screening project of the National Cancer Institute along with skatole and indole-3-acetic acid. All three were injected subcutaneously and found not to be carcinogenic. However, Storti found that it could induce leukemia.[54,48]

It was found that either indole or skatole injected in a 1/100,000 dilution decreases the response of mouse intestinal muscle to acetycholine. Tryptamine slightly increases this effect while all three inhibit the automatic movements of the duodenum. Serotonin and gramine have an inconsistant effect on blood pressure but cause uterine contraction in cats.[53,9]

Wooley et. al. tested indole derivatives for their action in causing constriction of the carotid artery. Serotonin, present in salivary glands and gastrointestinal tissues of mammals and in the platelets of mammalian blood, is the vasoconstrictor substance which causes the above mentioned phenomena. Tryptamine, 5-amino and 5-mitro tryptamine, and 3-(B-dimethylaminoethyl)5-aminoindole were found to have a similar action. The 3-alkyl 5-aminoindoles and 2,3 dialkyl-5-aminoindoles were found to be antagonists.[59]

Many properties of pyrrole are similar to indole except that pyrrole is less toxic,[5] the mid-lethal dose being 6g./kilogran as opposed to 60 mg./kilogram for indole.[24] For

the ketopyrroles this mid-lethal dose increases in the order: pyrroylmethyl ketone less than ethyl, less than propyl, less than isobutyl.[29] The isobutyl derivative causes convulsions whereas the ethyl does not.[28]

All of the above mentioned derivatives cause a disappearance of reflex action and are depressents to respiratory movements at lethal concentrations. Isovalerylpyrrole is a narcotic to mice.[28]

2,4 Dimethyl-3-pyrrole carboxylate causes motor phenomena and convulsions. It raises blood pressure while arterial hypertension and respiration is weakened. Sodium-5-acetyl-2,4-dimethyl-3-pyrrole-carboxylate is less toxic but at high concentrations gives complete paralysis.[12]

Elderfield's Organic Reactions states that pyrrole, in all its reactions, is more reactive than indole.[16]

The polymerization of indole has been found to be catalysed by hydrogen ions and is not dependent upon the acid present. Pyrrole is much more liable to polymerization and is even polymerized in the presence of glycols.[49]

Metabolism of Indoles[1]

The metabolism of tryptophan is presented here in schematic form as an example of general indole metabolism. There are two major metabolic pathways. In the cell tryptophan is successively hydroxylated to 5-hydroxytryptophan, decarboxylated to serotonin, and oxidized to 5-hydroxyindoleacetic acid, which is excreted. The second major metabolic pathway is through formylkynurenine, which is formed through oxidation by oxygen gas in the presence of a liver enzyme. It has been established by tracer studies using radioactive oxygen gas and not from the water present. Formylkynurenine is rapidly hydrolysed to kynurenine by another enzyme, also present in the liver. Kynurenine is converted in insects into ommochrome, a pigment, while in mammals it is excreted as such, and as anthranilic, kynurenic, and xanthurenic acids. Kynurenine can also be converted enzymatically into nicotinic acid, one of the B group of vitamins. (See Figure II).

Figure II (Part 1)

The Metabolism of Indole

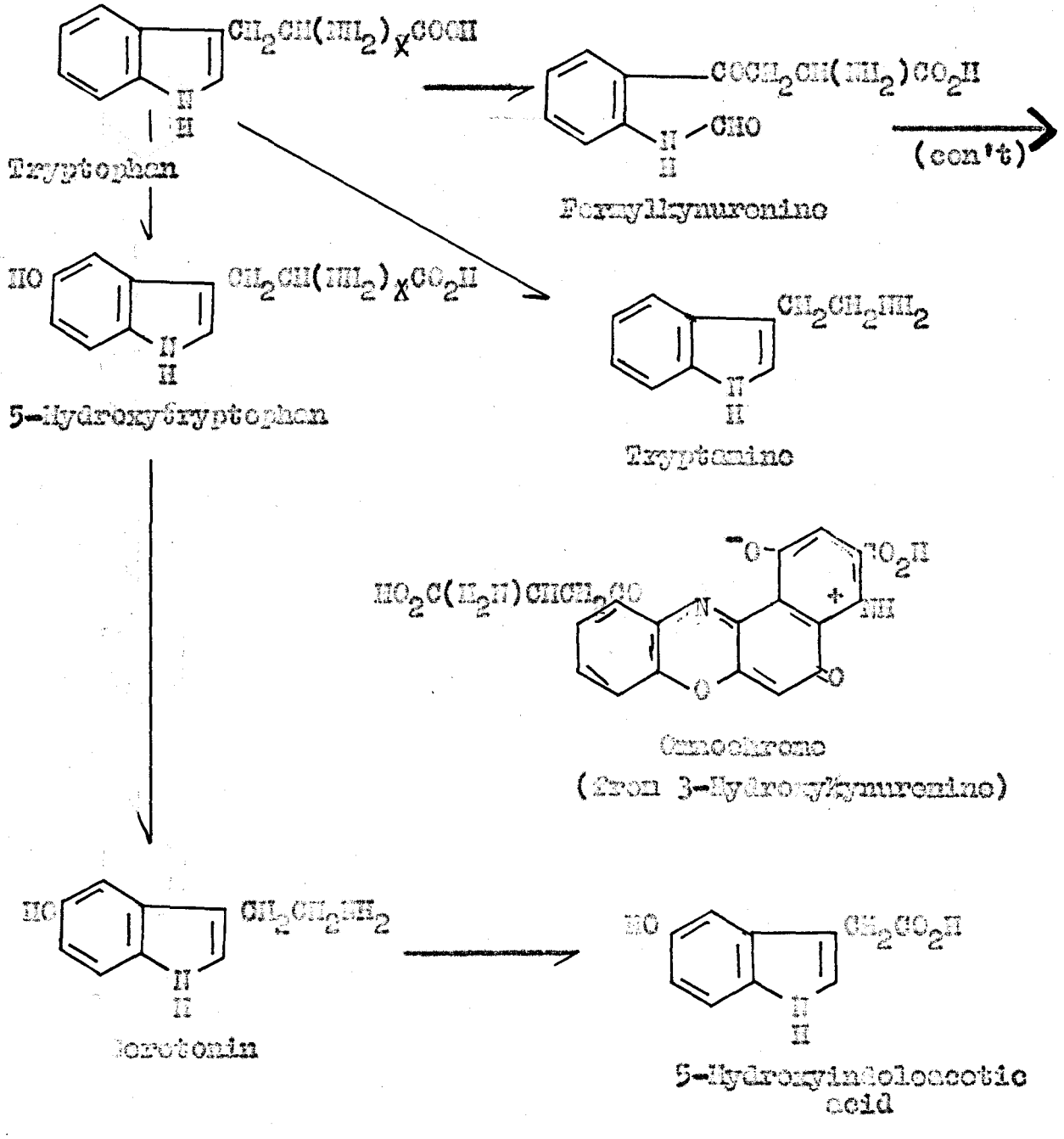
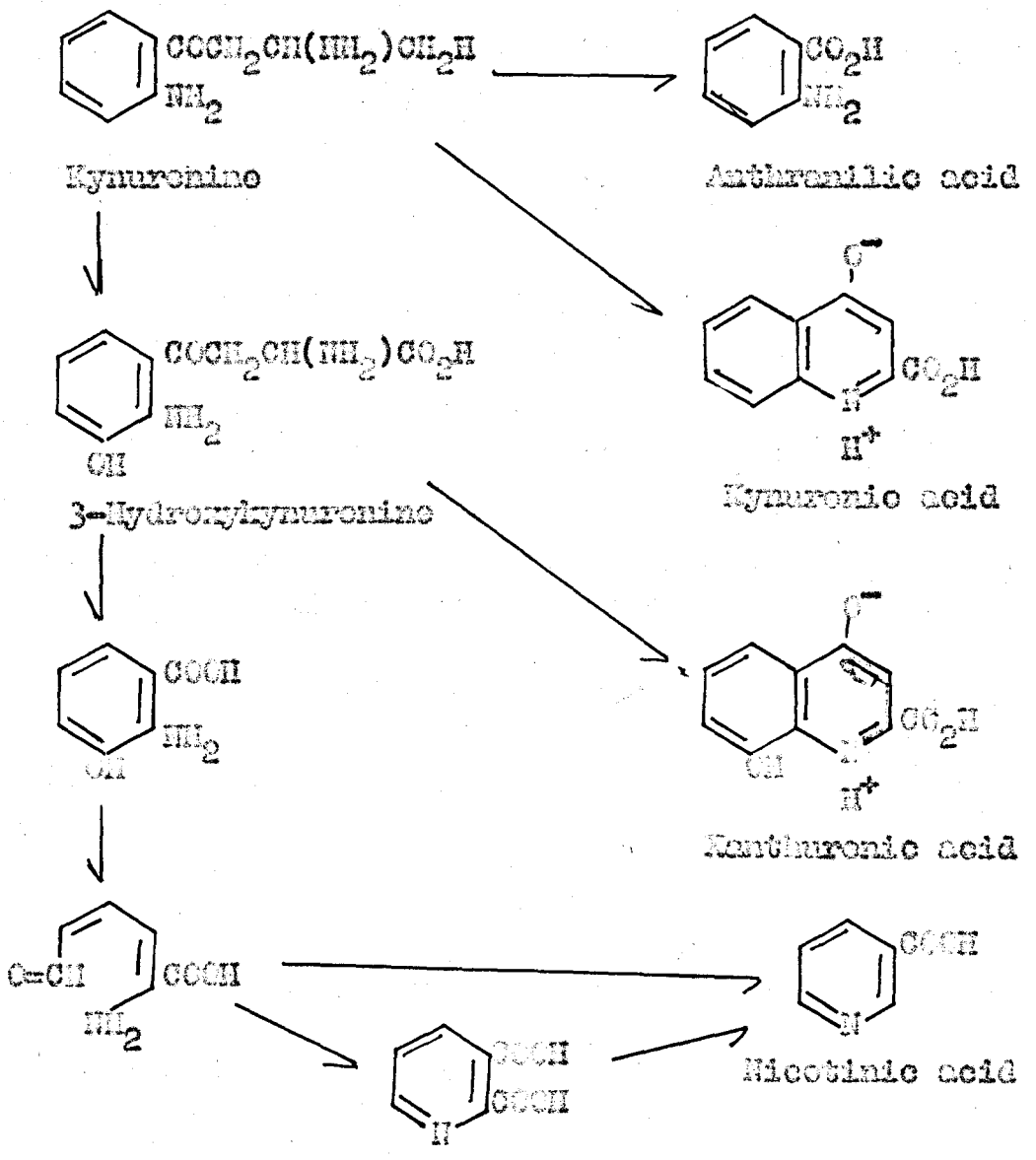


Figure II (Part 2)

The Metabolism of Indole



Preparation of Methane Sulfonates and Nitrogen Mustard Alkylating Agents

There are three general methods for the preparation of Methane Sulfonates.

(1) Refluxing an alcohol with Methane Sulfonic Acid to give poor yields.[2]

$$ROH + CH_3SO_3H \rightleftharpoons ROSO_2CH_3 + HOH$$

(2) Refluxing Methane Sulfonyl chloride with an alcohol, or reacting in pyridine.[32]

$$ROH + CH_3SO_2Cl \longrightarrow ROSO_2CH_3$$

(3) Refluxing the silver salt of Methane Sulfonic Acid with an alkyl chloride.[10,52]

$$RCH_2Cl + CH_3SO_3Ag \longrightarrow RCH_2OSO_2CH_3 + AgCl$$

There are four general methods for the preparation of Nitrogen-Mustards.

(1) Chlorination of OH with $SOCl_2$[30]

$$RN(CH_2CH_2OH)_2 + SOCl_2 \longrightarrow RN(CH_2CH_2Cl)_2 \cdot HCl$$

(2) Mannich Type reactions[27]

$$RH + RN(CH_2CH_2Cl)_2 + CH_2O \longrightarrow RCH_2N(CH_2CH_2Cl)_2$$

(3) Elimination of HCl from an amine and an alkyl halide[31]

$$RCl + NH(CH_2CH_2Cl)_2 \longrightarrow RN(CH_2CH_2Cl)_2 \cdot HCl$$

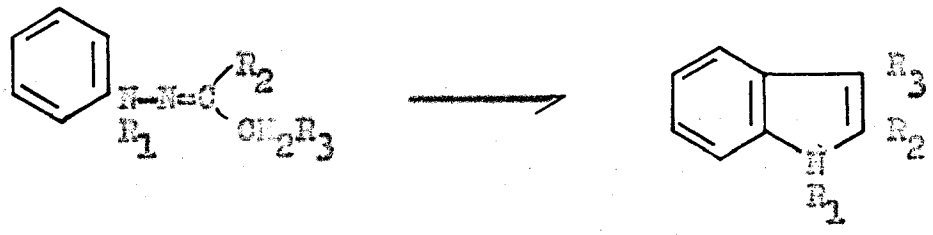
(4) Reduction of amides with $LiAlH_4$, or $NaBH_4 + AlCl_3$[6]

$$R\overset{O}{\overset{\|}{C}}N(CH_2CH_2Cl)_2 \xrightarrow{LiAlH_4} RCH_2N(CH_2CH_2Cl)_2$$

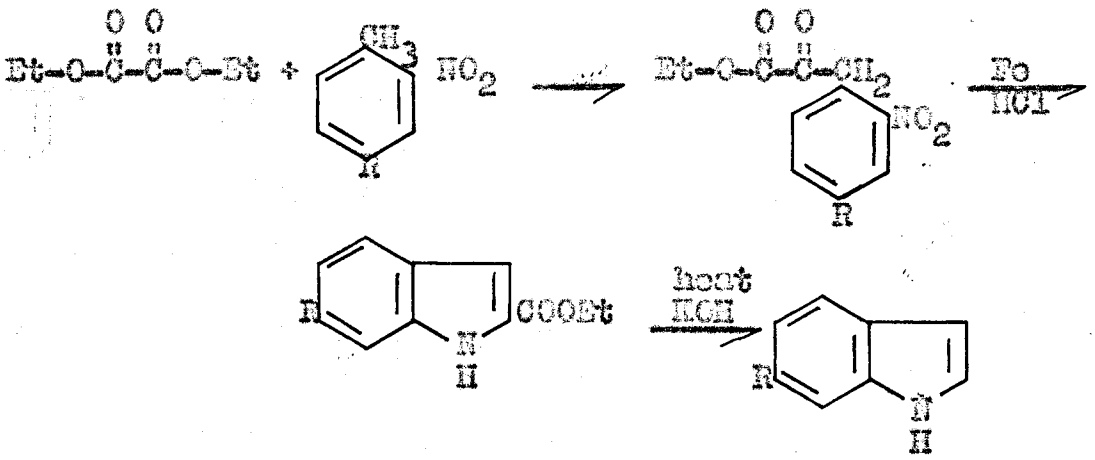
$$R\overset{O}{\overset{\|}{C}}N(CH_2CH_2Cl)_2 \xrightarrow{AlBH_4} RCH_2N(CH_2CH_2Cl)_2$$

The Synthesis of Indole and Pyrrole Derivatives

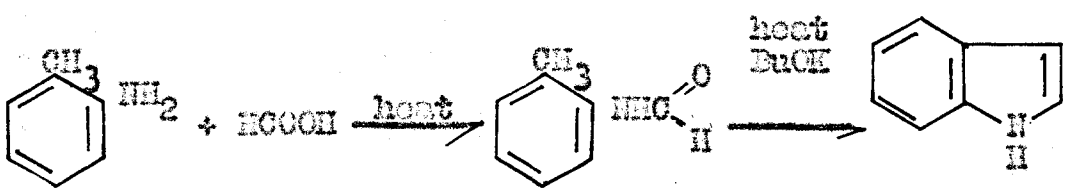
Probably the most familiar indole synthesis is the well known Fisher Indole synthesis where a phenylhydrazone is heated over zinc chloride at 160°.[14]



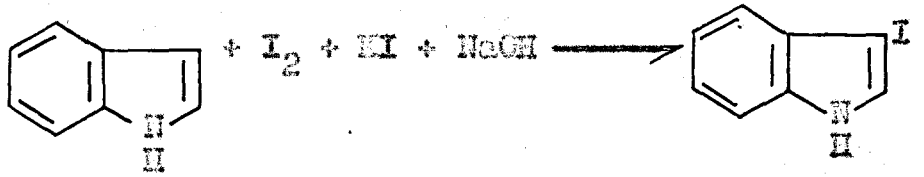
Ethyl oxalate may be condensed with a substituted nitrotoluene, the product reduced, and the ring closed.[58]

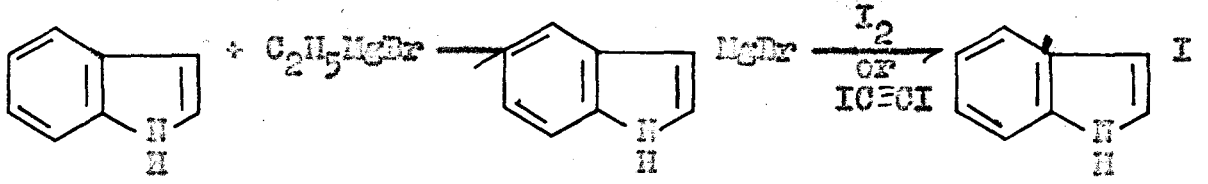


A similar synthesis may be through an o formyltoluide[51]

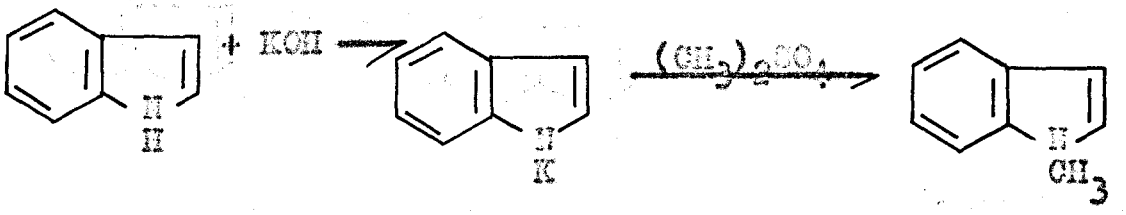


Indole may be halogenated easily in the three position by addition of halogen in CH_3CH[3] or substitution of a Grignard reagent.[17]

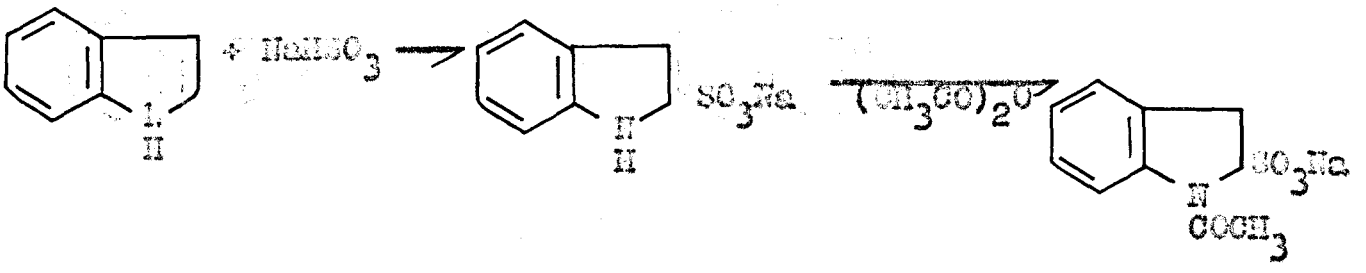


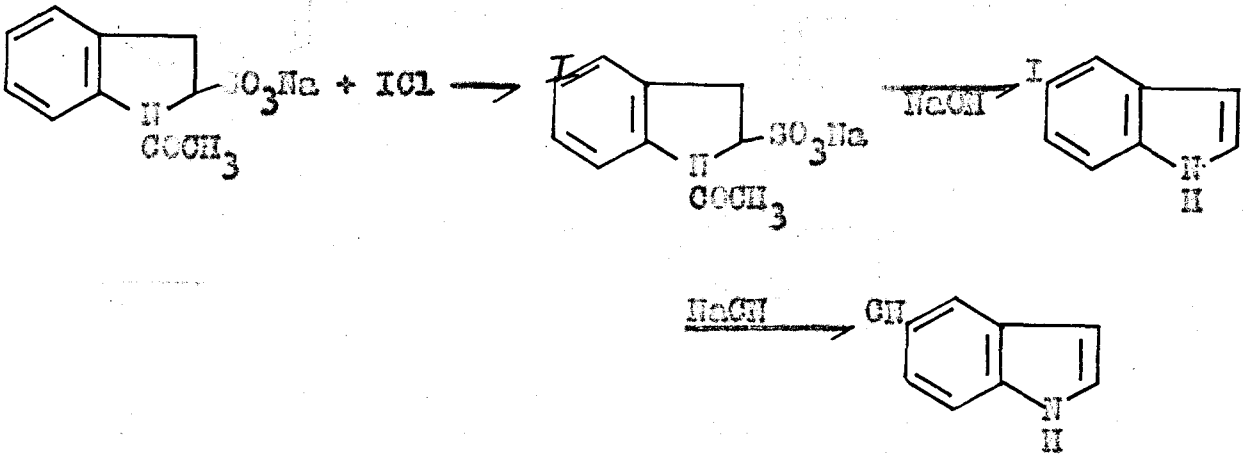


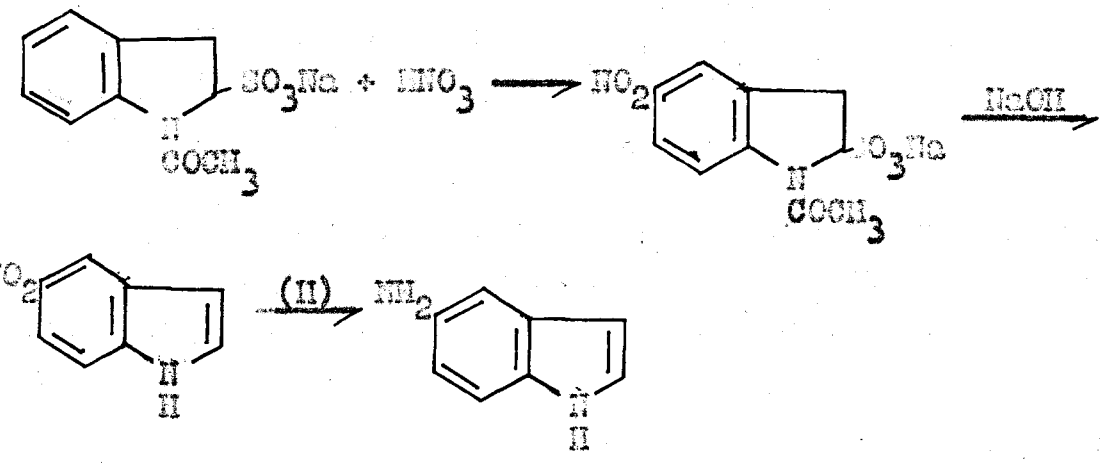
Indole may be substituted in the one position by forming the potassium salt and then reacting with an alkyl halide or sulfate.[21]



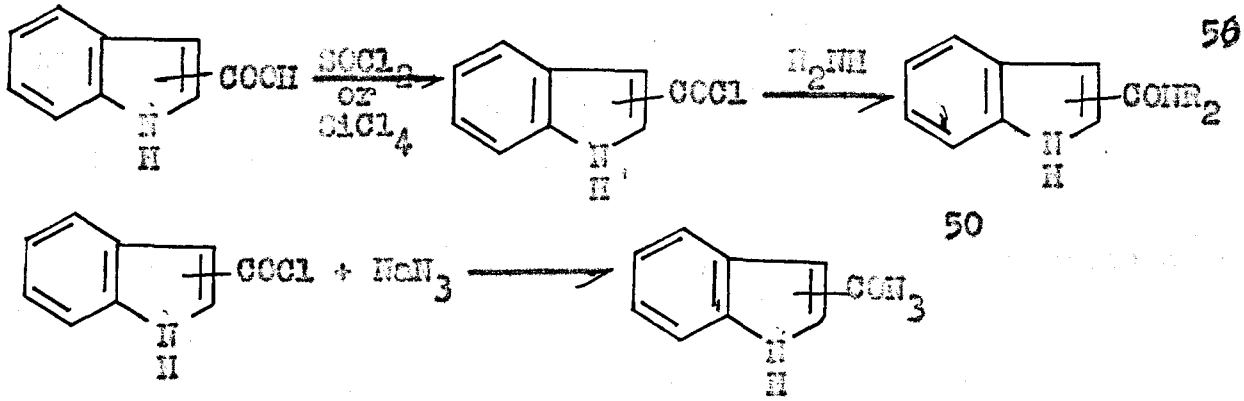
An interesting method for making 3-substituted indoles is by condensation with $NaHSO_3$ to make the Indolylsodiumsulfonate, and acetylation. The resulting product has many of the properties of acetanilide. The indole is recovered with 20% NaOH.[57]

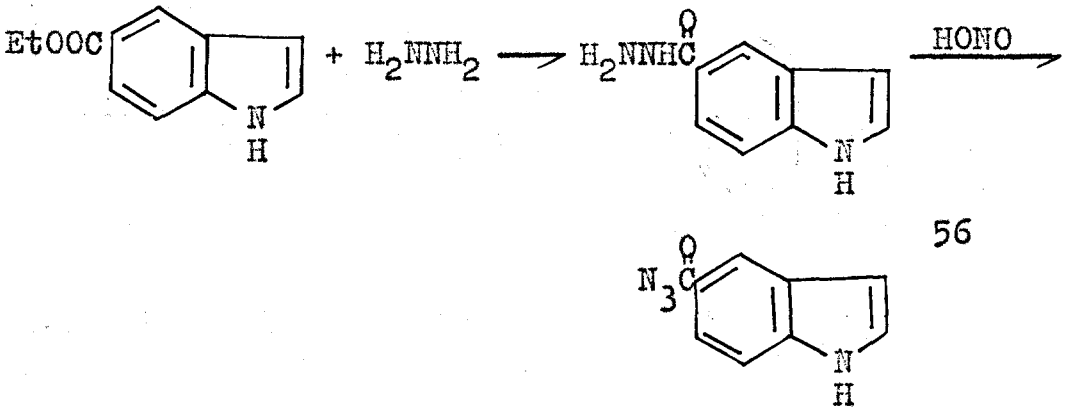






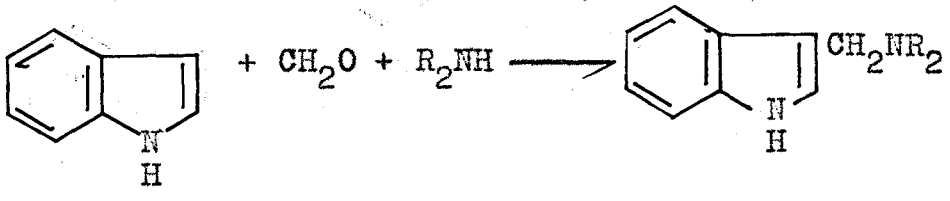
Acid chlorides and amides may be prepared through routine methods and reacted with secondary amines.



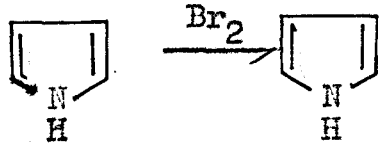


56

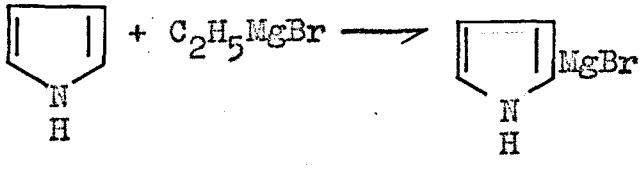
Indole also undergoes the Mannich reaction.[15]



Pyrrole may be reacted with a halide to give a polyfunctional derivative or with a Grignard reagent giving 2-substituted derivatives, which have the normal reactions of a Grignard reagent.[13]



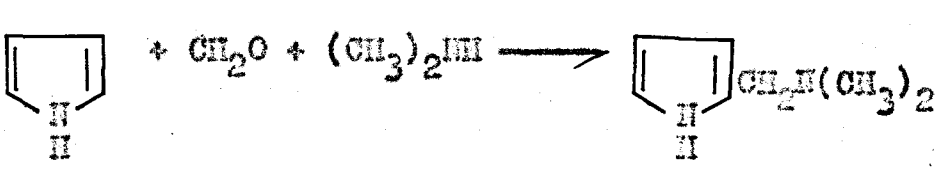
Br , also di-, tri-, and tetra derivatives.



etc.

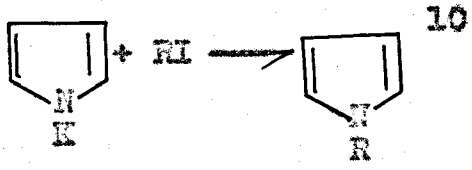
Like indole, pyrrole may undergo the Mannich reaction one

or more times.



4

N-Substituted derivatives may be obtained by reactions similar to those with indole.



10

EXPERIMENTAL

Theory

Evidence has been given (p.11) for the establishment of a pH differential between normal tissue and tumors in the body. Just about all methods taking advantage of this variant have been centered around protection of the normal tissues from the action of amines, with alkylating groups attached, possessing proper pKa values(p.13). It is herein asserted by the author, that, if a compound could be found which would be precipitated over a pH of 6.9-6.4 and not precipated at a pH of 7.0-7.2, of normal body pH, this could be used as a tumor specific agent. It will be shown that indole, an analogue of pyrrole, satisfies that criterion.

Therefore, an alkylating substituent is placed on a pyrrole nucleus in the 2-position and on an indole nucleus in the 2, 3, or 5 position. The compound, administered in aqueous glucose suspension, should be selectively precipitated or polymerized in the walls of tumor cells. The alkylating substituent should then become permanently attached to the cell wall, probably the lipid constituents, thereby changing its permeability and causing the tumor cells to suffer respiration failure. Any unpolymerized indole or pyrrole based drugs not precipitated should be detoxified by the liver.

Experimental Evidence for the Selective Deposition of Indole in Mammary Gland Tumors of the C3H Mouse

To test the applicability of using the variant of pH lowering to cause an organic compound to be selectively deposited in or on malignant cells, it was decided ~~upon~~ to purchase indole labled with carbon 14 and determine it's location in a mouse's body at various time intervals. The reason for chosing indole was that it is known to be subject to acid catalyzed polymerization but is stable in basic solution.

Indole Assay Method I
(utilization of carbon 14 labled molecules)

Equipment and materials

Indole-2-C^{14} picrate
C3H mice with and without mammary tumors
Saturated aqueous glucose solution
Hypodermic needles
Apparatus for drying and pulverizing tissues
Flow counter equipped with a 0.9 mg/cm^2 gold window
Baird-Atomic model 132 scaler
Pyrex planchet 3/8 inch in diameter

Procedure

Indole-2-C^{14} picrate was dissolved in 10% sodium hydroxide solution and extracted with ligroin until there was but negligible radiation in the aqueous layer. The organic layer was evaporated and the indole suspended in 0.5 ml. of saturated aqueous glucose solution.

A female C3H mouse, with a one-half inch diameter mammary tumor, was allowed to feed on a solution made up of one part water and one part saturated aqueous glucose solution for one-half hour after being starved for four hours.

The indole suspension was then administered to the mouse by intraperitoneal injection. After the specified time interval shown in the tables, the animal was sacrificed, the desired tissues dried *in vacuo*, pulverized, placed upon the planchet at constant thickness, and the radiation counted for one minute in the flow counter. Background count, determined just before counting, was subtracted before the data was entered.

Results

One preliminary run was made with 0.1 millicurie of radiation to determine whether or not further work was desirable. Muscle, from comparable muscle tissue in which the tumor was growing, and the tumor itself were tested. A count of 5091/min. was obtained from the tumor and 12/min. from muscle tissue after an incubation time of four hours, indicating that the theory was workable and needed further investigation.

In one run, 0.1 ml. of indole-2-C^{14} picrate was divided into eight doses. These results are shown in Table I.

In another run, 0.2 mc. of indole-2-C^{14} picrate was divided into nine doses and activities measured for 12, 24, and 48 hours. These results are shown in Table 2.

Table 3 shows a test run over periods of 48 and 72 hours with 0.1 mc. of indole-2-C^{14} picrate divided among five mice.

The activities between various mice cannot be compared quantitatively in these tables, since it was impossible to administer exactly equal doses of the indole suspension to each mouse.

Table I

Mouse No.	1	2	3	4	5	6	7
Time (hrs.)	0	2	3	3.5	12	12	4
Tumor	1456	270	170	70	58	84	260
Muscle	1172 756	16	20	8	0	0	0
Blood*	11.2	14	2	3	.5	1.5	4
Urine*		150	108	10	10	0	0
Brain		0	50	0	0	0	10
Liver		46	66	36	20	60	28
Kidney		210	400	16	0	36	6
Stomach		38	110	14	22	0	0
Lung		78	17	36	20	58	10

*—0.5 ml. urine mixed with 0.045 g. brain tissue and dried. Recorded as counts/min./0.5 ml. urine.

*—Dried blood was weighed and mixed with 0.5 g. brain tissue. Recorded as counts/min./mg. blood.

Mouse #1 was a control. The tissues were taken from a

mouse and 0.025 ml. indole suspension added to each. Mouse #7 contained the Erlich Ascites tumor and is not included in further data tabulations. The tumor cells were obtained by centrifugation.

Table II

Mouse No.	1	2	3	4	5	6	7	8	9
Time (hrs.)	48	12	12	12	12	12	24	24	48
Tumor		680	700	1745	1415	267	117	160	804
Muscle	0	0	0	0	0	0	0	0	0
Liver	92	560	710	2740	1508	150	146	110	83

Mouse #1 was used as a control. It had no tumor and only the liver was counted

Table III

Mouse No.	1	2	3	4	5
Time (hrs.)	48	48	72	72	72
Tumor	130	111	23	0	14
Muscle	0	0	0	0	0
Liver	120	90	10	0	11

Interpretation of Results

Though the data are not adjusted for statistical fluctuations in counting, an overall look at Table I, excluding values for the tumors, shows the normal body functions. One can see the detoxified indole being excreted through the kidney into the urine with all organs, except the liver, decreasing in indole concentration with respect to time. Note however, that the tumor absorbs as much or more indole than the liver and/or kidney, and, that the radiation content of the tumor does not decrease as rapidly as in the other body organs. Tables II and III indicate that some indole is present in the tumor for 72 hours.

It is easily seen that indole remains in the tumor, even though all other organs except the liver lose their indole content within a few hours. The high isotope content of the tumor cannot be due to a large amount of blood trapped in the tumor since the indole content of the blood is negligible after three hours.

The high radioactive count of the liver must at first be due to the detoxification of the large amount of indole which was injected into the mouse, with the exception of that indole which was absorbed and retained by the tumor. Then, slowly, over a 12 hour period, most of that indole is metabolized out of the tumor to be passed into and detoxified by the liver.

If indole were polymerized in the fluid around the tumor cells, it would be rapidly removed by the blood stream. The data argues against this since the radiation level of the blood is so low at all times, whereas the radiation level in the tumor remains relatively high for more than 12 hours. Therefore, the indole is probably polymerized either inside the cell itself or in the cell wall as the historical portion of this

thesis (p. 15) indicate.

The most important data, in the author's opinion, are the values of the indole content of the tumor versus the indole content of the muscle. The fact that the indole content of the muscle is always so small indicates that the pH induction of the tumor has indeed been used to cause a selective deposition. To the author's knowledge, this is the first instance that such an effect has been observed.

Resulting Hypothesis

Nitrogen mustards and alkyl methanesulfonates will react with the hydrophobic constituents of a living cell in considerably less than twelve hours, which is the time limit for the maximum concentration of indole as shown in the tables. Therefore, the indole moiety should act as an effective transport molecule for alkylating agents to be used as antineoplastic drugs. The alkylating substituent should combine with the cell wall, if indole concentrates there, and change its character enough to inhibit cell respiration, thereby killing the cell through respiration failure. If indole concentrates elsewhere in the cell, the alkylating substituent should interrupt metabolic pathways resulting in the death of the cell.

Diagnostic Use of Radioactive Indole

It was suggested that if a gamma ray emitter were placed upon an indole molecule, the resulting molecule might serve as a locator for tumors. To investigate this idea, 5-iodoindole was synthesized[57] containing iodine-125.

Synthesis of 5-I^{125}-indole

A solution of 70 ml. of ether, 50 g. of indole, 150 ml. of 40% sodium bisulfite, 50 ml. of water and 300 ml. of ethyl alcohol was shaken for 10 hours, yielding 70 g. of sodium-2-indolinosulfonate. Fifteen g. of this product and 150 ml. of acetic anhydride was stirred at 50° C. for three hours and filtered to give the N-acetyl derivative.

Five mc. of iodine 125 was diluted to 5.6 g. with reagent grade iodine. This product was added to 1.72 g. chlorine to yield 7.37 g. of iodine monochloride.

To 15 ml. of water and 3.0 g. of N-acetyl-sodium-2-indolinosulfonate, the iodine monochloride was added during 10 minutes. The reaction mixture was stirred at 0° C. for one hour and at room temperature for one hour. Fifty ml. of water was added and the solution was filtered. The filtrate was made basic with 20% sodium hydroxide, cooled for an hour, and 1.98 g. (52.4%) of the title product was obtained by filtration.

Procedure for testing diagnostic possibilities:

Into a solution of 6 ml. of polyethylene glycol 300 and 4 ml. of saturated aqueous glucose was dissolved 0.10 g. of 5-I^{125}-indole and 0.5 ml. of the resulting solution was injected into two C3H mice with no tumors and three C3H mice with tumors.

Results:

The radiation was measured with a ratemeter focused upon the tumor and upon the abdomen from a distance of one inch. The resulting data are shown in Table IV. The numbers stand for readings on the ratemeter. The figure ∞ means that the count rate was too high to measure by this method.

	Body radiation					Tumor radiation		
Mouse No.	1	2	3	4	5	3	4	5
Time (hrs.)								
2.5	∞	∞	∞	∞	∞	800	900	700
3.5	∞	∞	∞	900	950	∞	900	∞
4.5	1000	1000	900	600	∞	900	900	∞
5.5	1000	1000	900	700	∞	∞	900	∞
6.5	died	died	900	700	∞	900	∞	∞
10.0			died	died	died			

Mice numbers 1 and 2 had no tumors and were used as controls.

Interpretation of results:

From the data above, no conclusion can be drawn. From other tests in which indole derivatives concentrate in tumor cells, the author feels that smaller doses of 5-I^{125}-indole with higher concentrations of radioactive iodine could have diagnostic uses.

INDOLE ASSAY METHOD II

Other Indole Derivatives Concentrating in Tumors of the C3H Mouse

By a color test method to be described in a thesis to be presented by Mr. Ronald Floyd, the following compounds have also been found to concentrate in tumors of treated C3H mice:

Gramine
Indole-3-carbinol
Tryptophol
Sodium-N-tryptamine-gamma-propane sulfonate
2-Methylindole
3-Methylindole
7-Methylindole
3-Indolebutyric acid
N-Beta hydroxyethyl-2,3,4,5-tetramethylpyrrole

The following compounds gave slightly positive or doubtful tests:

3-Indolepropionic acid
5-Bromoindole
5-Hydroxyindole
5-Aminoindole
2-Indoline sodium sulfonate
1-Acetyl-2-Indoline sodium sulfonate
Pyrrole

The following compounds were shown not to concentrate in treated mice using the above procedure:

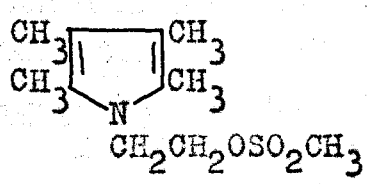
2-Indolecarboxylic acid
3-Indolecarboxylic acid
5-Indolecarboxylic acid
1-Indoleacetic acid

3-Indoleacetic acid
2-Indolecarboxylic acid diethylamide
5-Indolecarboxylic acid diethylamide
3-Indolecarboxaldehyde
5-Hydroxy-3-indoleacetic acid
Pyrrole-2-carboxaldehyde
Furan

2-C^{14}-2-Indoleacetic acid was found not to concentrate in tumors of treated mice using the same procedure as for the assay method for 2-C^{14}-indole.

Synthetic Methods

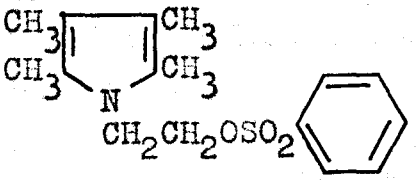
1-(2-Methanesulfonoxyethyl)-2,3,4,5-tetramethylpyrrole



To a solution of 7.41 g. (0.03 mole) N-beta hydroxyethyl-2,3,4,5-tetramethyl pyrrole in 4.8 ml. of pyridine was added 4.6 g. (0.04 mole) of methanesulfonylchloride at 0-10° C. The reaction mixture was then stirred keeping the temperature below 30° C. As soon as the crystaline material separated, the reaction mixture was poured into 200 ml. ice water and washed twice with water. The title product was obtained as 9.16 g. (93.4%) of white crystals, m.p. 65° (decomp.). Molecular weight. For $C_{11}H_{19}NO_3S$: 247. Found, 260,320.

The freezing point depression of diphenyl was used for the first molecular weight determination and acetic acid for the second.

1-(2-Benzenesulfonoxyethyl)-2,3,4,5-tetramethylpyrrole

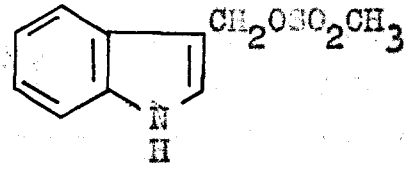


To a solution of 7.41 g. (0.03 mole) of N-beta hydroxyethyl-2,3,4,5-tetramethylpyrrole in 4.8 ml. of pyridine was

added 7.08 g. (0.04 mole) of benzenesulfonylchloride at 0-10° C. The reaction mixture was then allowed to stand at room temperature for one-half hour, poured into 200 ml. of ice water. The crystals obtained were washed with 20 ml. of methanol, yielding 8.5 g. (72%) of the white, crystalline product, m.p., 74° (decomp.). Molecular weight. For $C_{16}H_{21}NO_3S$: 309. Found, 346, 375.

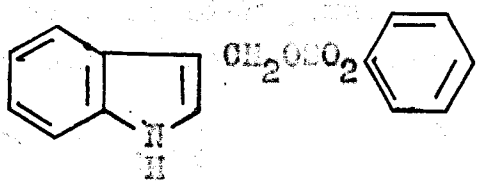
The freezing point depression of diphenyl was used to determine the first molecular weight and acetic acid to determine the latter.

3-(Methanesulfonoxymethyl)indole



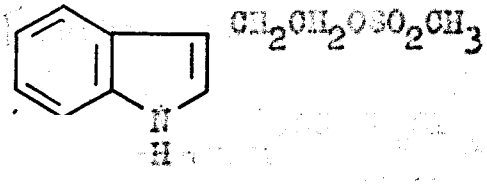
To a solution of 30 ml. of pyridine and 4.44 g. (0.03 mole) of indole-3-carbinol was added 4.60 g. (0.04 mole) of methanesulfonylchloride at 0-10° C. The reaction mixture was allowed to stand at 0° C. for three hours, and was then poured into 400 ml. of ice water. This reaction mixture was extracted with 100 ml. ether. The ether extract was washed with water until all pyridine was removed, then dried, and evaporated. The title product was obtained as 6.7 g. (97.7%) of a dark unstable oil.

3-(Benzenesulfonoxymethyl)indole



To a solution of 30 ml. pyridine and 4.44 g. (0.03 mole) of indole-3-carbinol was added 7.08 g. (0.04 mole) of benzenesulfonylchloride at 0-10° C. The reaction mixture was allowed to stand at 0° C. for three hours and was then poured into 400 ml. of ice water. The reaction mixture was extracted with 100 ml. ether and the ether layer washed until all pyridine was removed. The extraction was then dried and evaporated. The title product was obtained as 8.53 g. (90.1%) of a dark unstable oil.

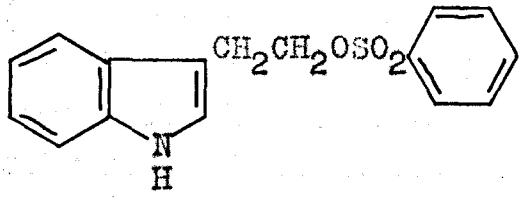
3-(2-Methanesulfonoxyethyl)indole



To a solution of 3.0 ml. pyridine and 2.0 g. (0.012 mole) of tryptophol was added 1.72 g. (0.015 mole) of methanesulfonylchloride at 0-10° C. The reaction mixture was allowed to stand at room temperature for one-half hour and was then poured into 400 ml. of ice water. The resulting oil was washed twice with water and dried, yielding 2.7 g. (93.9%) of the viscous oily product. Molecular weight. Calculated for

$C_{11}H_{13}NO_3S$: 277. Found, 224.

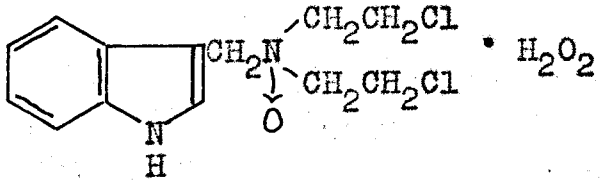
The freezing point depression of acetic acid was used to determine molecular weight.



To a solution of 3.0 ml. of pyridine and 2.0 g. (0.012 mole) of tryptophol was added 2.65 g. (0.015 mole) of benzenesulfonyl-chloride at 0-10° C. The reaction mixture was allowed to stand at room temperature for one hour and was then poured into 400 ml. of ice water. The resulting oil was washed twice with water and dried, yielding 3.21 g. (95.2%) of the viscous oily product. Molecular weight. For $C_{16}H_{15}NO_3S$: 301. Found, 293.

The freezing point depression of acetic acid was used to determine molecular weight.

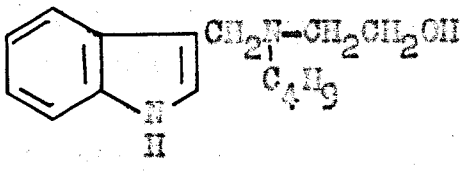
3-[Bis(beta chloroethyl)aminomethyl] indole • H_2O_2



To 50 ml. of a saturated K_2CO_3 solution was added 7.08 g. (0.04 mole) of bis-(2-chloroethyl) amine hydrochloride and the free base was extracted immediately with 20 ml. of ether. The ether extract was added to 9 ml. of glacial acetic acid and the

ether evaporated *in vacuo* below 30° C. This mixture was then cooled to 0-10° C. and treated with 5 ml. of 35% formaldehyde and 4.56 g. (0.04 mole) indole. The reaction mixture was kept below 30° C. for 30 minutes and then poured into ice water. (At this point product isolation was attempted as the hydroacetate, but the product was shown to be unstable upon concentration.) The organic layer was dissolved in 200 ml. 95% ethylalcohol and 20 ml. of 30% hydrogen peroxide added. More alcohol was added to effect solution and the mixture made basic to pH paper with alcoholic potassium hydroxide. After stirring overnight at room temperature, an equal volume of tert-butyl alcohol was added and 0.6 g. (4.04%) of the title product was obtained as white crystals, with no definite melting point, by cooling and filtration. Calculated for $C_{13}H_{18}Cl_2N_2O_3$: Cl, 22.11%. Found, 21.83%.

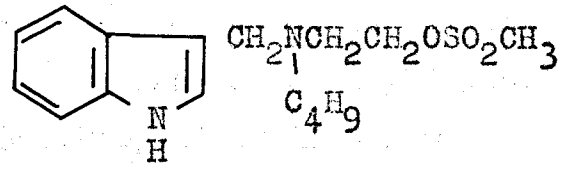
3-(Butylethanolaminomethyl)indole



To 30 g. (0.2 mole) of glacial acetic acid at 0° C, 11.2 g. (0.1 mole) butylethanolamine was added. Twenty ml. (0.2 mole) of 30% formaldehyde was added and immediately thereafter 15 g. (0.13 mole) indole. After one hour at room temperature the mixture was triturated with ether until all the excess indole was removed. The aqueous layer was neutralized with 20% aqueous sodium hydroxide and extracted with ether. The Mannich

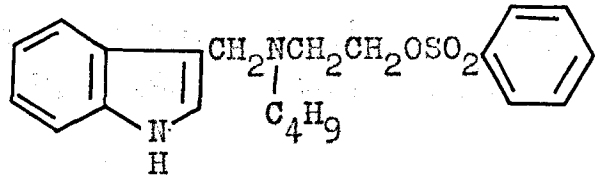
base, a light colored oil, was obtained by evaporation of the dried etheral extract.

3-(N-Butyl-N-Methanesulfonoxyethylaminomethyl)indole



To 7.34 g. (0.03 mole) of 3-(butylethanolaminomethyl)indole dissolved in 8 ml. pyridine was added 4.56 g. (0.04 mole) of methanesulfonylchloride with the temperature kept at 0-10° C. After addition was complete, the mixture was brought to room temperature and allowed to stand for thirty minutes. The mixture was poured into 400 ml. of ice water containing 3.2 g. (0.08 mole) of sodium hydroxide. The oily product thus formed was washed twice with water and dried, yielding 30.3 g. (91.2%) of the viscous, oily product. Calculated for $C_{16}H_{24}N_2O_3S$: Titrateable N, 4.26%. Found, 4.16%.

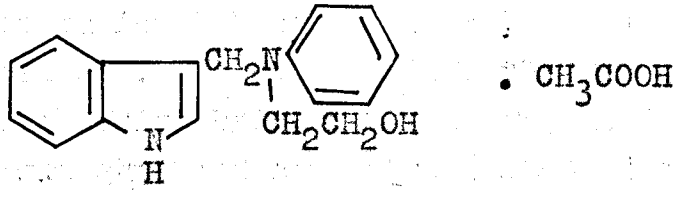
3-(N-Butyl-N-benzenesulfonoxyethylaminomethyl)indole



To 7.34 g. (0.03 mole) of 3-(butylethanolaminomethyl)indole dissolved in 8 ml. pyridine was added 4.50 g. (0.4 mole) of benzenesulfonylchloride with the temperature kept at 0-10° C.

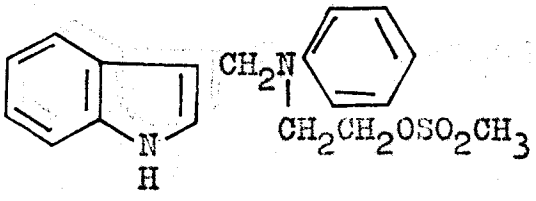
After addition was complete, the mixture was brought to room temperature and allowed to stand for one hour. The mixture was poured into 400 ml. of ice water containing 3.2 g. (0.04 mole) of sodium hydroxide. The oily product formed was washed twice with water and dried, yielding 6.24 g. (94.8%) of a viscous, oil. Calculated for $C_{21}H_{26}N_2O_3S$: Titrateable nitrogen, 3.73%. Found, 3.51%.

3-(Phenylethanolaminomethyl)indole hydroacetate



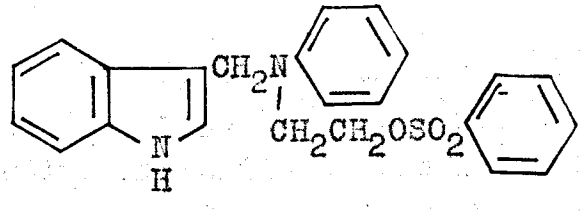
To a solution of 60 ml. of acetic acid, 13.7 g. (0.1 mole) of phenylethanolamine and 20 ml. of 30% aqueous formaldehyde at 0° C. was added dropwise a solution of 17.55 g. (0.15 mole) of indole in 10 ml. of acetic acid, keeping the temperature below 10° C. After addition was complete, the reaction mixture was allowed to stand at room temperature for one hour. The resulting solid material was triturated with ether until the excess indole was removed. The solid was then dried. The title product was obtained as 31.0 g. (99%) light pink crystals, m.p. 184° (decomp.). For $C_{19}H_{22}N_2O_3$: titratable N, 4.49%. Found, 4.45%.

3-(N-Phenyl-N-methanesulfonoxyethylaminomethyl)indole



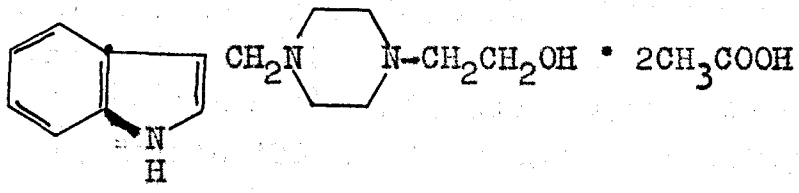
A mixture of 6.30 g. (0.02 mole) 3-(phenylethanolaminomethyl)indole, 60 ml. of pyridine and 2.88 g. (0.025 mole) of methanesulfonylchloride was allowed to stand overnight at room temperature. To the reaction mixture was added 50 ml. of 20% aqueous sodium hydroxide and 400 ml. ice water. The title product was obtained as 6.9 g. (100%) of white crystals, m.p. 194°, by filtration. For $C_{18}H_{19}N_2O_3S$: titrateable N, 4.06%. Found, 4.54%.

3-(N-Phenyl-N-benzenesulfonoxyethylaminomethyl)indole



A mixture of 6.30 g. (0.02 mole) of 3-(phenylethanolaminomethyl)indole, 60 ml. of pyridine and 4.4 g. (0.025 mole) of benzenesulfonylchloride was allowed to stand overnight at room temperature. To the reaction mixture was added 50 ml. of 20% aqueous sodium hydroxide and 400 ml. ice water. The title product was obtained as 8.1 g. (100%) of white crystals, m.p. 194° C. by filtration. For $C_{23}H_{22}N_2O_3S$: titrateable N, 3.42%. Found, 3.98%.

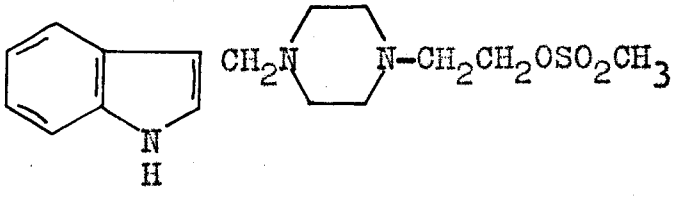
3-[4-(Hydroxyethyl)piperazinomethyl]indole dihydroacetate



To a solution of 12 ml. acetic acid, 13.1 g. (0.1 mole) of N-hydroxyethylpiperazine and 20 ml. of 30% aqueous formaldehyde at 0° C. was added 17.55 g. (0.15 mole) indole keeping the temperature below 20°. After addition was complete, the reaction mixture was allowed to stand at room temperature for one hour. The resulting solution was triturated with ether until the excess indole was removed. A viscous oil was obtained over which was poured 200 ml. ice water with slow stirring. The title product was obtained as 6.5 g. (17.3%) of white crystals, m.p. 57°. For $C_{19}H_{29}N_3O_5$: titrateable N, 7.38%. Found, 7.56%.

The free base of the title product was obtained as 10.2 g. (39.3%), m.p. 71°, of white crystals by addition of 20% aqueous sodium hydroxide to the filtrate from which the title product was obtained. For $C_{15}H_{21}N_3O$: titrateable N, 10.81%. Found, 10.93%.

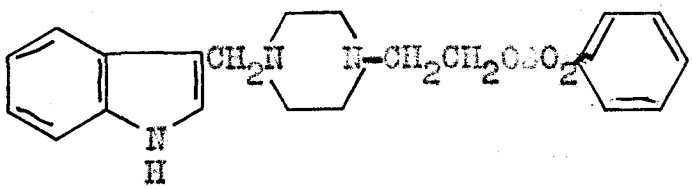
3-[4-(Methanesulfonoxyethyl)piperazinomethyl]indole



To 3.78 g. (0.01 mole) 3-[4-(hydroxyethyl)piperazinomethyl

indole] dihydroacetate in 10 ml. of pyridine was added 1.7 g. (0.015 mole) of methanesulfonylchloride, keeping the temperature at 0-10° C. After addition was complete, the reaction mixture was allowed to come to room temperature and was poured into 400 ml. ice water containing 1.2 g. (0.03 mole) of sodium hydroxide. The title product was obtained as 2.8 g. (83.2%) of a fine white solid, m.p. 122 (decomp.), by filtration. For $C_{16}H_{23}N_3O_3S$: titrateable N, 8.33%. Found, 7.92%.

3-[4-(Benzenesulfonoxyethyl)piperazinomethyl]indole



To 2.57 g. (0.01 mole) of 3-[4-(hydroxyethyl)piperazinomethyl]indole in 8 ml. pyridine was added 2.64 g. (0.015 mole) benzenesulfonylchloride keeping the temperature at 0-10° C. The reaction mixture was allowed to stand at room temperature for one-half hour and poured into 400 ml. of ice water containing 1.2 g. (0.03 mole) of sodium hydroxide. The oil obtained was dried <u>in vacuo</u> yielding 3.6 g. (87.3%) of the title product as a yellow solid, m.p. 37° C. For $C_{21}H_{25}N_3O_3S$: titrateable N, 7.19%. Found, 6.57%.

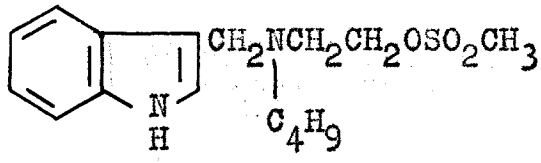
Derivatives of 3-Methylindole

All of the before mentioned indole derivatives have been in the 3-position of indole. Since skatole (3-methylindole) also concentrates in tumors of the C3H mouse 2-substituted derivatives of skatole may be of chemotheraputic value. Preliminary reactions indicate that Mannich bases may be introduced into the 2-position of skatole.

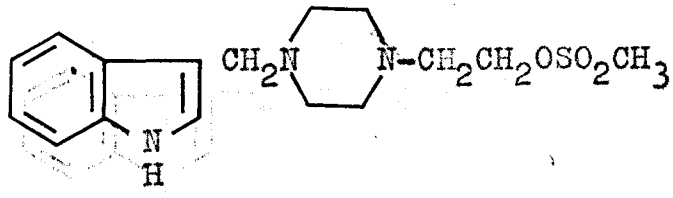
Analytical

Titration methods

The purity of some of the compounds prepared in this work was determined by titration of the basic nitrogen atom(s) using glacial acetic acid as solvent and 0.1 N perchloric acid in glacial acetic acid as the titrant.[49a] The following compounds, for example, would contain:



one titrateable nitrogen atom



two titrateable nitrogen atoms

Equipment: Beckman Glass Electrode pH meter, Model H-2.

Reagents: Reagent grade glacial acetic acid, 0.1 N perchloric acid in glacial acetic acid.

Procedure: A sample ranging from 0.05 *g.*-0.1 *g.* is weighed into a 250 ml. beaker. The sample is dissolved in 25-35 ml. of glacial acetic acid. Using a pH meter to follow the change in potential of the solution, the sample is titrated with 0.1 N perchloric acid in acetic acid. Increments of 0.10 ml. are added to the solution and the endpoint is indicated

by the greatest change in potential per 0.10 ml. increment of acid.

Calculations:

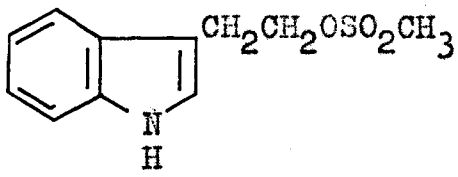
$$\frac{\text{A x Wt. sample x 1000}}{\text{ml. Acid x N acid}} = \text{Molecular Weight}$$

A=Number of titrateable nitrogen atoms

$$\frac{\text{14.0 x A x 100}}{\text{Molecular Weight}} = \%\text{Nitrogen}$$

Freezing point depression method

In compounds prepared which contained no basic nitrogen atom, for example,



molecular weights were determined by the freezing point depression method.[23a]

As a consequence of the lowering of the vapor pressure by addition of a nonvolatile nonelectrolyte, the freezing point is depressed. Then,

$$M = Kf\left(\frac{1000\ g_2}{g_1\ \Delta T}\right.$$

where M= molecular weight

Kf = molal freezing point constant

ΔT = observed lowering of freezing point

g_1 = grams solvent

g_2 = grams solute.

Gravimetric method

Nitrogen mustards react readily with silver methanesulfonate according to the following equation.[18, 15]

$$RN(CH_2CH_2Cl)_2 + 2CH_3SO_3Ag \longrightarrow RN(CH_2CH_2SO_3CH)_2 + 2AgCl$$

Calculations:

$$\%Cl = \frac{\text{g. ppt. (A) X 100}}{\text{g. sample}}$$

$$A = \text{gravimetric factor} = \frac{\text{at. wt. Cl}}{\text{mol. wt. AgCl}}$$

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Autobiography

I, Andrew G. Bachmann was born in East Orange, New Jersey, on January 12, 1940. In June of 1958, I was graduated from Warwick High School in Newport News, Virginia. In September of 1958, I entered the University of Richmond and received the Bachelor of Science degree in August of 1963.

I entered the Graduate School of the University of Richmond in September of 1963 and was awarded a laboratory Assistantship for the school terms of 1963-1964 and 1964-1965, and the summer terms of 1964 and 1965. I was Puryear Fellow in Chemistry for the second term 1964-1965. During the school term of 1964-1965 and during the summer of 1965, I have done research on designing drugs which will be selectively deposited in cancer cells with Dr. J. Stanton Pierce.