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*I hereby recommend that the thesis prepared under my supervision by* Sidney Schulman

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# SYNTHESIS OF POTENTIAL CARCINOGENS
# AND
# CANCER THERAPEUTIC AGENTS

A dissertation submitted to the

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by

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# INTRODUCTION

To man probably the most striking phenomenon of nature is life. Although he has speculated through the ages on the origin and reproduction of life, yet only recently have we begun to consider one of its most essential constituents, growth. Because man is so familiar with the growth cycle he seldom takes time to reflect upon it.

By processes of cell multiplication and differentiation a complete organism is formed from a single fertilized cell. Yet why does man achieve one particular size and stop enlarging? Though cell differentiation is practically completed at birth, body enlargement occurs for quite some time and then suddenly stops. Although size is limited in man, growth continues throughout life. However, after maturity, growth takes place only in a regenerative capacity. In fact as old age occurs even this rate is diminished and man becomes somewhat smaller in size.

## Growth and Tumors

Tumors may be defined as (1): "an abnormal growth of cells, not inflammatory, but arising without obvious cause from cells of preexistent tissue, possessing no physiological function." This type of growth is frequently unlimited, without consideration for neighboring tissues which it progressively invades, destroys, and replaces, heedless of the well-being of the organism as a whole. This disorder is common to all

living multicellular forms, both plants and animals. Its presence has been observed for thousands of years. Clinical observations of cancer have been recorded as early as 1500 B.C. (2).

Tumors arise from cells in the host by an irreversible transformation of unknown etiology. Most adult mammalian tissues cultured "in vitro" fail to grow except during a brief early period, whereas most cancerous tissues under the same conditions exhibit continuous growth over many years. (Tumorous tissue therefore seems to show a capacity for growth not inherent in normal tissues.) Even "in vivo", if normal tissues are transplanted to other sites in the organism they may be maintained briefly, but will eventually atropy and die. However, cancerous tissue cell masses actually invade other tissues. These detach themselves from the original cancer and are deposited in distant organs by means of the circulatory system. At these sites new cancers grow. This is known as metastosis.

Although cancers grow more rapidly than the normal cell in its regenerative equilibrium, malignant tumors do not grow as fast as cells which are being replaced due to injury to the tissue. It is known that yellow fever destroys liver tissue, and it has been found that dogs which lost 150 to 200 grams of tissue by this disease can regenerate this material in ten days to two weeks (3). This is indeed a much more rapid rate than the many years it often takes a cancer to reach similar proportions.

There seems to be a gradual loss in structural differentiation in metastatic tumors (4). They become more anaplastic, i.e., they resemble to a decreasing degree the normal tissues of their origin, so that occasionally metastatic growths from primary tumors of different kinds of normal tissues are indistinguishable. Like the primary tumor, the metastatic growths continue progressively, sometimes at a considerably more rapid rate than the primary tumor. Although the presence of these transplanted cells are incompatible with the harmonious balance of the organism, the organism does not seem to have any effective ability to stop these growths.

## CLASSIFICATION OF TUMORS

Occasionally, a tissue may grow in localized areas a little beyond the ordinary architectual scheme of the organism. As long as the growth remains under the control of the organism, this overgrowth usually occurs relatively slowly, and the effected tissue may ultimately either return to its normal size or at some time achieve a balance and stop growing. These limited abnormal growths are common and do not cause any harm to the organism as a whole, unless of course they impinge by mechanical force on some vital area of the body (such as nerve or cranial tissue). Their specific physiological functions as well as their microscopic cell patterns resemble largely those of the tissues from which they originated. These are known as benign tumors.

Malignant tumors or cancers are those growths over which the organism has apparently lost control. It is parasitic to the organism and performs no function but to overwhelm and destroy adjacent functioning tissues. The microscopic appearance varies considerably, but usually resembles in certain features either the tissues or embryonic, primitive forms of the tissues from which they originated. The cell patterns are frequently disordered, degenerated, and undifferentiated.

The malignant tumors are further classified according to the normal tissue from which they arise or most resemble. A carcinoma is that malignant tumor which has arisen from

epithelial tissue (either from the epithelium or the epidermus). The term sarcoma is that malignant tumor which has arisen from connective or muscle tissue. Thus a cancer of fibrous connective tissue would be termed a fibrosarcoma, and one from lymphatic tissue a lymphosarcoma.

## CARCINOGENS

Spontaneous animal tumors have been only infrequently employed because of the incomplete knowledge prevailing, until recently, as to the conditions necessary for their production in quantities required for experimental research. Because of this, it became desirable to devise some means of experimental tumor production in animals which would as closely as possible resemble those found in man. The persistant efforts in this direction finally met with success through the work of Yamagima, and Itchekawa (5). In 1915 these investigators first announced that they had caused the development of true cancerous growths in rabbits by applying coal tar to the ear of the animals for a long period of time. This discovery led to a new era in experimental cancer research, leading to an ever increasing number of findings, and the disclosure of many new problems.

In designating a compound as carcinogenic or non-carcinogenic, it is necessary to have a wide range of information. The carcinogenic action of a substance is known to be influenced by many factors including the genitic constitution of the animal (species and strain), its age and sex, diet, the physical condition of the animal, the purity of the compound, the nature of the solvent or vehicle used in administration, and the route or site of application. In addition, the value of the results is dependent upon the number of animals used, their survival rate, and the duration of the experiment.

Thus the appearance of tumors is dependent to a high degree upon the experimental conditions, while the number of tumors and the rate of their appearance are subject to many modifying influences.

At present only fragmentary evidence can be drawn between molecular structure and carcinogenic activity. But the fact that we are unable to place the entire number of potent chemical compounds in a unified theory of the cause of cancer far from indicates the absence of such correlations, but points to the ignorance of our basic understanding of the problem. Since the nature of these correlations is yet conjective, further study in this field may possibly lead to fruitful results.

## Coal Tar and the Hydrocarbons

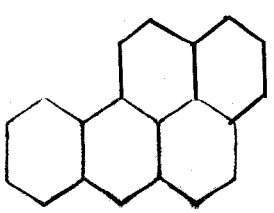
The scrotal cancer of chimney sweeps is the oldest recognized cause of an occupational tumor development. This disease was first described by the English physician Percival Pott (6) and has been repeatedly studied, especially in England, where the greatest amount of clinical material was available. With the development of the coal tar industry during the 19th century an increased incident of skin cancer was also noticed on workers in this industry.

The first stepping stone in the isolation of effective carcinogenic agents in the form of pure and structurally

defined substances was the observation that the greatest part of the carcinogenic activity of coal tar was contained in the fractions boiling at higher temperatures. However, none of the then known chemical constituents of these fractions could be demonstrated as tumor producing (7).

To help elucidate the problem, industrial tar production was imitated in two ways. First simple organic molecules were subjected to temperatures between 720° and 920° C, and next tetralin was allowed to be acted upon by aluminum chloride. In both cases high boiling aromatic hydrocarbons were obtained which induced malignant tumors in experimental animals (8). But the most significant observation was that these synthetic tars, as well as the natural ones, exhibited characteristic fluorescent spectra, with well defined bands at wave lengths of 4000, 4180, and 4400 angstrom.

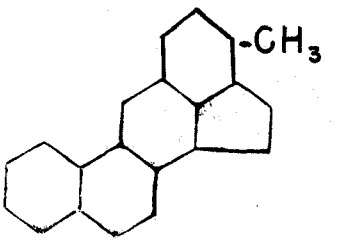
In 1933 Cook, Hewett, and Hieger (9) succeeded in isolating, in pure form, from coal tar a hitherto unknown hydrocarbon whose structure was established by synthesis as 3,4-benzpyrene. This compound was powerfully carcinogenic and possessed a fluorescent spectrum identical with that



3,4-Benzpyrene

of the active fractions of the original tars. Although 3,4-benzpyrene is probably the most active carcinogenic agent in coal tar, other hydrocarbons have been synthesized which also possess carcinogenic properties (10).

The most active one thus far synthesized and most widely used in experimental carcinogenesis is methyl cholanthrene.

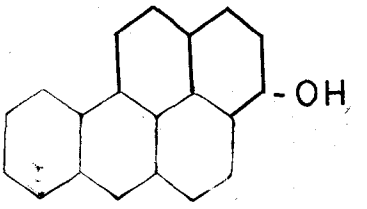



**Methyl Cholanthrene**

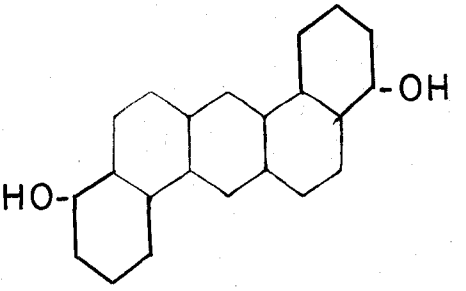
In order to shed some light on the mechanism of tumor formation, the metabolic fate of the hydrocarbons was studied. By following the known fluorescence spectra in the ultraviolet some concept of the early fate of hydrocarbons at the site of application could be followed. Most of the hydrocarbons, per se, disappeared relatively quickly.from the sites of application (11). However, their disappearance may be due to some transformation of the hydrocarbon applied, or to some alternative product of which they are a part. The method used for their detection, i.e. their fluorescent spectra

in ultra-violet light, is limited because their spectrum could be altered by a multitude of changes.

The fate of hydrocarbons applied was partially answered by the examination of the excretory products of the animals tested. These studies show that the carcinogenic hydrocarbons are at least partially converted into phenolic derivatives. Thus 3,4-benzpyrene was converted to the mono-hydroxy derivatives (12), while 1,2,5,6-dibenzanthracene was converted to the dihydroxy derivative (13).

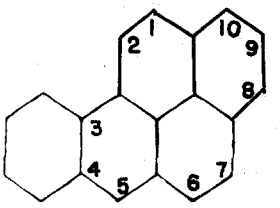



**7-Hydroxy - 3,4-Benzpyrene**

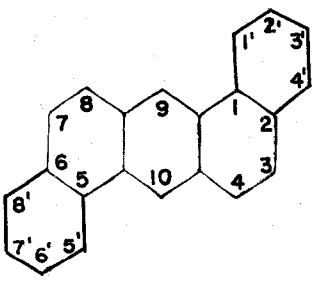



**4',8'-Dihydroxy - 1,2,5,6-Dibenzanthracene**

It is interesting to note that "in vitro" the usual most chemically active position in benzpyrene is 5-, and in dibenzanthracene the 9, 10- positions. When these positions are blocked, then the 8- position of 3,4-benzpyrene and the 4'8'- of 1,2,5,6-dibenzanthracene are most reactive (14).




**3,4-Benzpyrene**




**1,2,5,6-Dibenzanthracene**

None of the above mentioned hydroxylated substances possess carcinogenic activity. Only a relatively small percentage of the original hydrocarbon administered could be accounted for in the excretions. It therefore appears that the metabolism might unsuccessfully attempt to detoxify them. By the use of radioactive carbon the metabolism of the now missing portion may be investigated in the near future, shedding new knowledge on this problem.

Even upon one application of methyl cholanthrene to the epidermis, a series of changes have been observed to occur (15). The iron, calcium, cholesterol, and total lipid content of the skin decreased to about 50% of the normal within a few days. Continued applications further lowered the iron content, but the others mentioned remain at their initial abnormal level. While the copper and zinc content of the epidermis was also lowered, the magnesium content increased in this region. However, the sodium, potassium and ascorbic acid content were uneffected. Finally, when the cells became cancerous, there was a further lowering of all the components studied, except ion and ascorbic acid.

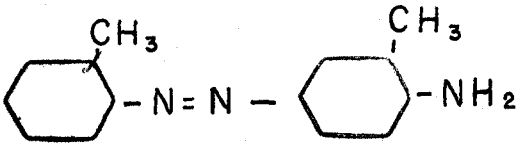
When mixtures of hydrocarbons possessing similar molecular structures but great differences in carcinogenic activity are applied the carcinogenic effect is weaker than if stronger agent had been applied alone. Thus it appears that the weaker agent acts in competition with the stronger agent, analgous to the sulfanilimide and p-aminobenzoic

acid competitive action.

## The Azo Dyes

The rapid development of the synthetic dye industry, inaugurated at the end of the 19th century was soon followed by the observation that workmen in these plants had a high frequency of bladder cancer. However, the early work utilizing dyestuffs produced only benign tumors which eventually regressed completely. About this time the carcinogenic action of coal tar was discovered and the activity of cancer research shifted to this field, with virtual abandonment of the dye carcinogens.

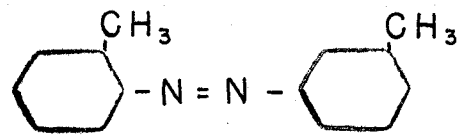
A new impetus was given to this phase when bladder tumors developed in rabbits who had been fed o-aminoazotoluene (16). In rats, either the oral administration of a 5% oily




**o-aminoazotoluene**

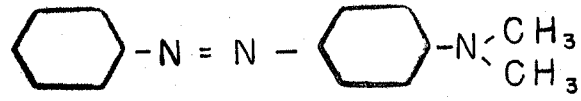
solution, or the injection of a 10% oily solution of o-aminoazotoluene produced malignant hepatomas (cancer of the liver)

and in some cases skin tumors were observed (17). The feeding of o,m'-dimethylazobenzene likewise resulted in the



**o-m'-dimethylazobenzene**

formation of bladder tumors (18). Among other azo compounds a dyestuff formerly used commercially for coloring butter and oils, p-dimethylaminozobenzene was found to be an effective

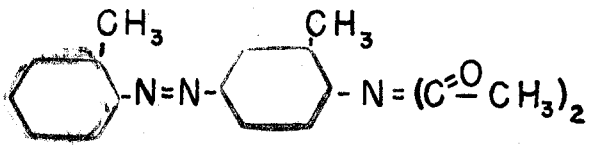


**p-dimethylaminozobenzene**

carcinogen by its production of hepatomas in rats (19). A series of compounds related to p-dimethylaminoazobenzene was studied by Suguira, Halter, Kensler and Rhoades (20), but

unfortunately, as with the hydrocarbons, no correlation between structure and carcinogenic ability could be found.

As with the carcinogenic hydrocarbons the metabolic fate of the azo dyes was studied. It was found that o-aminoazotoluene was excreted in the urine as the diacetyl derivative (21). When this metabolite was fed, it too turned out to



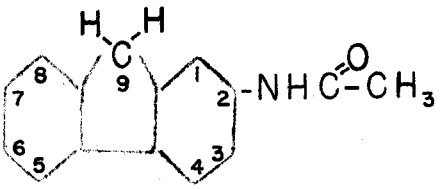
o-aminoazotoluene

be carcinogenic (22). It therefore appears that either the metabolism, in its attempt to detoxify the compound produces another carcinogen, or that the non-acetylated derivative, per se, is not carcinogenic, but that its metabolite is the active agent.

A closer metabolic study has been made on p-dimethylaminoazobenzene. Stevenson, Dobriner, and Rhods, fractionated the urine of rats which had been fed this dye (23). The isolation of p-phenylenediamine, p-aminophenol, and the acetylated forms of these substances led to the suggestion that the metabolic steps in the degradation of the dye was the splitting of the azo linkage, and demethylation. (That the reduction

of the azo to a hydrazo derivative takes place was suggested by the isolation of benzidine and aniline from the urine of rats fed azobenzene (24).) None of the products isolated from the urine, nor their respective acetylated forms could be shown to be carcinogenic when fed to rats. The experiments conducted for the search of metabolic pathways in the breakdown of azo dyes in the hope of correlating such mechanisms with that of carcinogenesis has been of great interest, but like its analogy with the polycyclic hydrocarbons, the work has yielded little, if any decisive information.

2-Acetamidofluorene

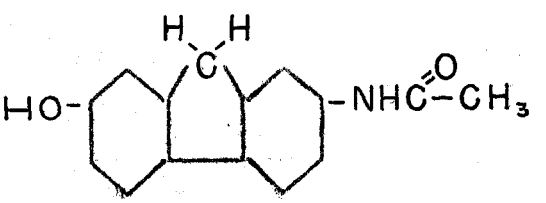



While investigating the toxic properties of 2-acetamidofluorene prior to tests as an insecticide, Wilson, De Eds, and Cox (25) observed that this fluorene derivative, when incorporated in the diet of rats, produced widespread tumor growth in 3-4 months. There are several points of interest connected with the carcinogenic activity of this material. The first point is that the compound must be fed orally, suggesting that

some metabolic reaction must be necessary to produce the actively carcinogenic derivative. The acetylated derivative is ineffective if placed under the skin in the form of a pellet, but the free base, when painted on the skin of rats, caused the production of hepatomas (26). The second point of interest is that no nitrogen compound as yet tested appears to induce such widespread and so many neoplastic lesions.

The simultaneous feeding of 2-acetamidofluorene and allyl thiourea resulted in the production of benign and malignant tumors in the thyroid gland of rats (27). The goiterous gland is apparently susceptible to the action of a carcinogen, which is inactive toward the unstimulated thyroid gland. When, however, 2-acetamidofluorene was fed before the allyl thiourea, only nodular goiter developed.

The metabolic fate of 2-acetamidofluorene was similar to that of the polycyclic hydrocarbons. In the urine of rats fed this amine, 2-acetamido-7-hydrofluorene was isolated (28). The 7-position represents the most active position "in vitro", after the 2-position has been occupied.




2-acetamido-7-hydrofluorene

## CHEMOTHERAPY OF TUMORS

In the strict sense of the meaning, the above mentioned title is a misnomer. Actually the title of this phase should be called the "Attempted Chemotherapy of Tumors", for as yet curative measures for tumors by chemotherapy is non-existant. With few exceptions, the work has been haphazard to the extreme and has been largely a groping in the dark. The objective has been principally based on endeavors to depress the rate of the malignant growth or create a milieu which would be unfavorable for the development of tumors. Many chemical agents are known which, when administered to tumor bearing animals, inhibit or even cause regression of the tumor growth. However, these substances are invariably toxic to the animals, and their seemingly beneficial effect may merely be due to the lack of nutrition supplied to the tumor because of total malnutrition in the body.

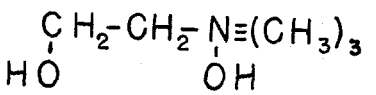
The ideal substance would of course be some material which is preferentially absorbed by malignant tumor cells, and which in the course of its metabolism would break down into agents toxic to the cell. Though this seems impossible today we may look at it philosophically that "The improbable we do immediately, but the impossible takes a little longer."

## The Cyanides

The injection of potassium cyanide in doses which provoke convulsions resulted in the complete regression of carcinoma in about 14% of the mice treated (29). The inhilation of hydrogen cyanide inhibited the growth of transplanted tumors, and even occasionally resulted in their complete regression. It was also found that the transplantability of hydrogen cyanide treated tumors was markedly impared (30). "In vitro", tumor slices showed as much as an 85-95% decrease in respiration, as well as an increase in radiosensitivity when treated with M/500-M/1000 solutions of potassium cyanide (31). On analogous behavior of radiosensitivity was noted "in vivo" when dilute potassium cyanide solutions were injected in mice afflicted with sarcoma (32). Generally, it is assumed that the tumor inhibiting effect of cyanides must be due to a severe respiratory disturbance, effecting the tumor cells in a greater degree than the normal cells. However the trouble with the use of the cyanides is that the ratio of the effective dose to the lethal dose is so close to one, as to make its use prohibitive.

## Choline

Trimethylhydroxyethyl ammonium hydroxide, a constituent of the lecithin molecule has also been tried.



**Choline**

Mouse tumors were found to recede following injections of this material, but no prolongation of life was observed, on account of the high toxicity of this substance. Better results were reported in animal experiments if salts of choline with iodobenzoic acid or glycine were used (33). However, later experimentation found choline administration to be ineffective, and choline therapy soon became obsolete.

## Benzene and Derivatives

The use of benzene initially attracted attention for lukemia because of its known ability to reduce the number of leucocytes. Administration of benzene to sarcoma-bearing rats has been reported as inhibiting the growth of tumors, at the same time however decreasing the radiosensitivity of the tumors (34). It was assumed that the leucocyte drop accompanying the benzene treatment might be responsible for the tumor inhibition, but on the other hand, the tumor in-

hibition may merely be the result of the expression of a general toxic effect of this compound.

The testing of almost one hundred different halogen substituted aromatic compounds demonstrated a growth inhibiting action of only tetrabromocresols, but clinical use of this substance was rejected because of the severe pains encountered in its administration.

A broader theoretical foundation appeared to be available for the investigation of nitrobenzene derivatives, particularly of dinitrophenols. These substances are known to increase the metabolic rate energetically, cause hyperthermia, and also inhibit cell mitosis (35). These substances also adversely effected tumor metabolism and increased the radiosensitivity "in vitro" (36). However, the administration of the dinitrophenols did not impede the growth of rat tumors, even in doses which increased the body temperature to 42°C and sublethal quantities failed to effect mouse sarcoma in spite of a considerable loss of body weight (37). Apparently the organism, in detoxifying the material, destroyed its action.

## Organic Dyes

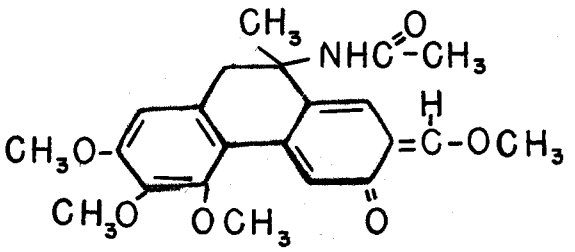
It was supposed for some time that some organic azo dyes, which intensively stained neoplastic tissue, might possibly destroy tumor cells or at least serve as vehicles in

transporting other tumor destroying agents to the cells. It has been shown, however, that the presupposed selective affinity of azo dyestuffs to tumor cells has not been proven for any of the substances tested, but that the stains were principally localized in the necrotic parts or in the connective tissue of the neoplasms (38). Therefore it appears evident as to the cause of failure of the action of azo dyestuffs either to limit tumor growth or to act as carriers for other substances which might limit tumor growth.

However, the use of fluorescent dyes for sensitizing tissue when exposed to suitable radiation has been used with moderate success. Some beneficial results were obtained when eosin was applied to skin tumors, and the tumors then subjected to the action of ultraviolet light (39).

## Mitotic Poisons

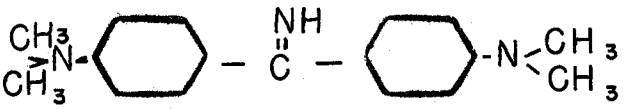
Very interesting results were obtained by A. P. Dustin and collaborators (40) in extensive histological investigations of colchicine on cells. This substance interferes with the normal course of cell mitosis, and thus arrests cell division. Although neoplastic cells are



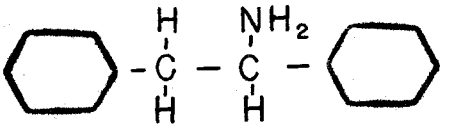
**Colchicine**

effected by this alkaloid, its effect is more pronounced on normal tissue (41). Many types of tumors failed to respond to colchicine therapy, and those that did respond required high doses of the drug. High doses of colchicine are toxic as they lack specificity and are damaging to the central nervous system. The practical usefulness of colchicine seems highly doubtful in view of the toxic doses necessary for achieving growth cessation of the tumor. Attempts to modify the chemical structure or obliterate the disadvantages while still retaining its beneficial effects on tumors has thus far proved fruitless.

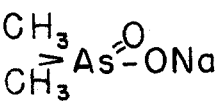
Other mitotic poisons, such as $\alpha,\beta$-diphenylethylamine, auramine and sodium cacodylate have been tried, but none has yet been found satisfactory.



**Auramine**



**α,β-Diphenylethylamine**



**Sodium Cacodylate**

## DISCUSSION

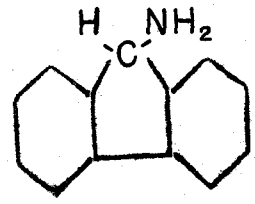
Although 2-aminofluorene and its N-acetylated derivative possess powerful carcinogenic activity, it is as yet impossible to explain the mechanism by which this occurs. Fluorene, per se, and 2-acetylfluprene possess no carcinogenic activity (42). Similarly, the acetamido group (which has been used in medicine for a long time in the form of acetanilid and its derivatives) has not been observed to produce neoplastic action. However, the combination of fluorene and the amino or acetamido groupings produce adverse physiological changes.

The margin between cancer producing and cancer inhibiting effects seems to be a matter of degree rather than kind. X-rays can cause neoplastic growths; on the other hand they can be used not only to inhibit these wild growths, but also to destroy them. Daudel (43) has shown that the carcinogenic action of certain very powerful cancer producing substances can be inhibited, but these substances which have hitherto inhibited this action have also possessed at least slight carcinogenic activity.

In view of these facts and in conjunction with previous work, the objective of this investigation was threefold. The first was to place the amino and acetamido groups in different positions on the fluorene molecule to ascertain whether merely the combination of these groups and the fluorene molecule were necessary for carcinogenic activity, or

substitution in the 2-position on essential requirement. As a sample test in this direction, the 9-position was chosen because of the ease of production of these derivatives.

Compound I was made in the following manner. Fluorene was oxidized to fluorenone by potassium dichromate in acetic acid solution.



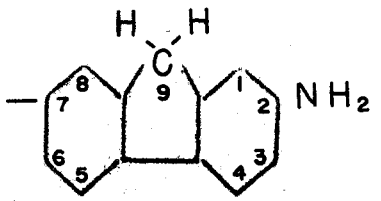

I

9-Aminofluorene

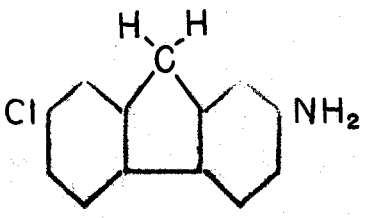
The ketone was then treated with hydroxylamine hydrochloride, in the presence of barium carbonate, and the oxime thus formed was reduced to the amine with zinc and acetic acid. The acetylated derivative was formed by the action of acetic anhydride on the amine.

F. Bielschowsky (44) has shown that 7-hydroxy-2-acetamidofluorene can be isolated from the urine of rats fed 2-acetamidofluorene. Therefore, if the organism does attempt to detoxify the material by this procedure, then substitution in that position should enhance the carcinogenic activity of 2-acetamidofluorene or produce a derivative

substituted in some other position. The problem was subdivided first by placing an inactive chlorine atom in the 7-position of 2-acetamidofluorene and 2-aminofluorene.



Then an amino or substituted amino group, potentially an active carcinogenic group, was placed in the 7-position of 2-acetamidofluorene. The 2-amino,7-chlorofluorene, II, was prepared by the chlorination of fluorene followed by nitration to yield 2-nitro,7-chlorofluorene. This was subsequently reduced to the amine by the action of zinc and calcium

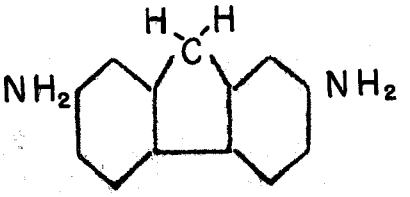


II

2-nitro,7-chlorofluorene

chloride. The N-acetylated derivative was produced by the action of acetic anhydride on the amine.

The 2,7-diaminofluorene, III, was synthesized by the reduction of 2,7-dinitrofluorene with tin and

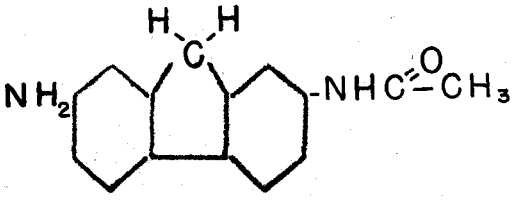


III

2,7-diaminofluorene

hydrochloric acid. The intermediate, 2,7-dinitrofluorene, was produced by the action of yellow fuming nitric acid on a semi refined grade of fluorene.

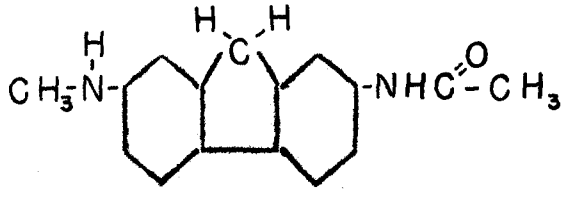
The monoacetylated diamine, IV, was formed by the



IV

2-acetamido-7-aminofluorene

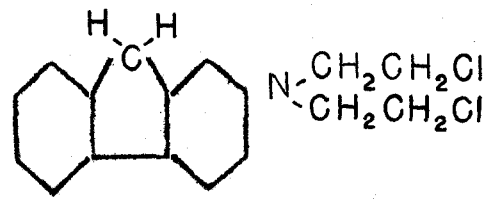
action of an equivalent amount of acetic anhydride on a cold aqueous solution of the diamine dihydrochloride salt, while the diacetamido derivative, V, was produced by the action of acetic anhydride on an acetic acid solution of the diamine. By the action of dimethyl sulfate on the monoacetylated derivative IV, the free amino group was monomethylated, yielding compound VI.



VI

**2-acetamido-7,N-methylaminofluorene**

The next objective was to prepare a series of compounds which might possess therapeutic activity. This series was also separated into two distinct divisions. In the first substituents were used which were already known to have some negative effect upon the growth of malignant tumors or upon growth itself. Nitrogen mustards are known to interfere seriously with tumor growth, but are thus far too toxic to the rest of the organism. In the search for a less toxic agent which would retain its effect upon tumor growth, compound VII was synthesized from 2-aminofluorene and

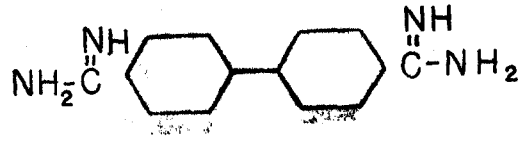


VII

**N,N,β-Dichlorethyl-2-Aminofluorene**

ethylene chlorohydrin.

The use of the amidino group has shown some definite physiological improvements in animals afflicted with malignant tumors, but so far most of its derivatives are rather toxic. Therefore a biphenyl derivative, VIII, similar in many respects to the fluorene molecule, was chosen due to the difficulty of production of the analogous fluorene derivative in quantities adequate for testing. The 4,4'-diazonium salt of

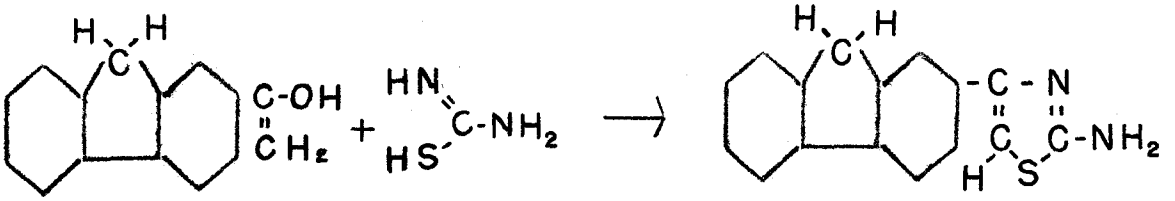


VIII

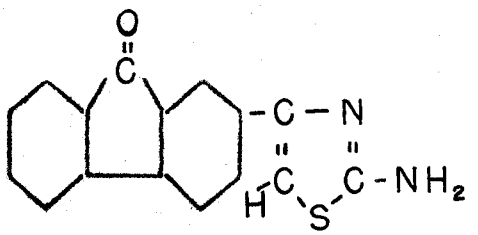
**4,4'-Diamidinobiphenyl**

biphenyl, made by the action of nitrous acid on benzidine, was added to an aqueous solution of potassium cyanide. The dicyanide derivative thus formed was treated with dry hydrogen chloride, in the presence of absolute methyl alcohol, forming a diiminoether. Dry ammonia gas converted the diiminoether into the diamidine.

The last objective of this work was to separate the fluorene ring and the attached amino group by a molecule of distinct physiological action. It was hoped that the organism might use this material preferentially, as in the sulfanilimide and benzoic acid equilibrium so successfully used against bacterial action. For this the thiazole ring was chosen. The condensation of 2-acetylfluorene or 2-acetylfluorenone with thiourea, in the presence of iodine yielded the thiazole derivatives used. The following shows schematically the reaction used.

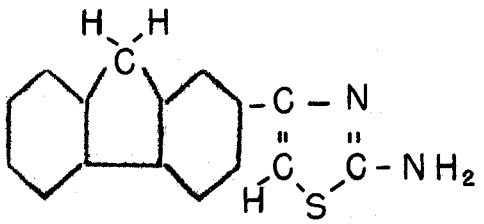


Compounds IX and X were prepared by this method, and compound XI was formed by the action of acetic anhydride on X.



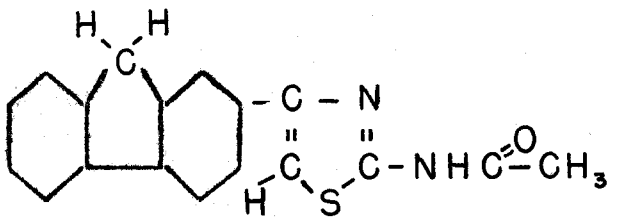
IX

4-(2-fluoryl-9-one)- 2-Aminothiazole



X

4-(2-fluoryl)- 2-Aminothiazole



XI

4-(2-fluoryl)- 2-Acetamidothiazole

## EXPERIMENTAL PROCEDURE

A. 2-Nitrofluorene (45) - The process used in Organic Synthesis has been improved upon in order to facilitate the temperature control and speed of production in the synthesis of 2-nitrofluorene.

To a 2-liter 3-neck flask, equipped with a Hirschburg mechanical stirrer, a thermometer, and a dropping funnel, was added 120 grams (0.72 M) of fluorene and one liter of glacial acetic acid. The mixture was stirred vigorously and heated until all the fluorene had dissolved. The temperature was adjusted to 60° - 65° and 160 ml. of concentrated nitric acid (sp. gr. 1.42) added in a slow steady stream. The temperature rose rather quickly and during this time the mixture was stirred rather vigorously. The temperature was not allowed to rise above 80°. A yellow precipitate formed and the solution turned to a paste. However, the paste was stirred vigorously to dissipate the heat, until the temperature was about 60°. (When the temperature began to fall the dropping funnel was replaced by suction in order to remove the oxidizing gases.) The product was filtered on a Buchner funnel, sucked as dry as possible, and then washed with two 50 ml. portions of glacial acetic acid, each containing one gram of potassium acetate, and then with one 50 ml. portion of glacial acetic acid. The 2-nitrofluorene was placed in a 2-liter beaker, covered with 1.5 liters of water, and stirred mechanically for a few minutes and filtered. The last washing was repeated

and the material air dried overnight. Yield 120 grams. m.p. 156° - 157°.

B. 2-Aminofluorene (45) - In a 2-liter round bottom flask equipped with a reflux condenser was added 30 grams of 2-nitrofluorene, 820 ml. of alcohol, 180 ml. of water, 10 grams of calcium chloride, and 300 grams of zinc dust. The mixture was refluxed for two hours, then filtered. The zinc remaining in the flask was extracted with 200 ml. of alcohol, filtered and the alcohol mixed with the filtered mother liquor. The filtrate was poured into three liters of ice water and filtered. Yield 20 grams. m.p. 126°.

The 2-aminofluorene can be recrystallized from 50% alcohol. m.p. 127°.

C. N, N, B-Dichlorethyl 2-Aminofluorene - Five grams of 2-aminofluorene and 50 ml. of ethylene chlorohydrin were refluxed together for six hours. Then 35 ml. of the solvent was distilled off and the resultant residue allowed to cool. To the residue was added 200 ml. of anhydrous ether, and a tan precipitate filtered. m.p. c.a. 170°. Yield 4 grams. After crystallization from acetophenone the precipitate melted at 180° - 193°. Recrystallization again from the same solvent, using Darco, turned the precipitate white, sintering at 186°, m.p. 193°. Subsequent recrystallization did not raise either the melting point or eliminate the sintering.

Anal. Calc. for $C_{17}H_{17}Cl_2N$: N, 4.58% Cl, 23.20%
Found: N, 4.96% 22.86%

D. 2,7-Dinitrofluorene (46) - In a 2-liter 3-neck flask, equipped with a mechanical stirrer, was placed 500 ml. of glacial acetic acid. To the stirred glacial acetic acid, 500 ml. of yellow fuming nitric acid (sp. gr. 1.52) was added in a slow steady stream. The temperature rose to 40° - 50° during this operation. Then 50 grams (0.30 M) of fluorene (m.p. 107° - 110°) was added in small increments to the warm solution, as the mixed acid was mechanically stirred. (Although the solution got quite hot, very little oxidation occurred.) The mixture was filtered hot through a sintered glass crucible and washed twice with a little glacial acetic acid. The precipitate was then placed in a 600 ml. beaker, covered with 400 ml. of water and stirred mechanically for ten minutes. It was then filtered and dried. Yield 45 g. Sintered 285°, m.p. 295°.

E. 2,5-Dinitrofluorene (47) - The mother liquor from the production of 2,7-dinitrofluorene was cooled, and a precipitate settled out. This was filtered and washed several times with glacial acetic acid, then with a little water. m.p. 195° - 205°. After recrystallization from glacial acetic acid the material melted at 207°.

The mother liquor was poured into three times its volume of water, filtered, and washed several times with water.

m.p. 180° - 195°. After several recrystallizations from glacial acetic acid, the material melted at 206° - 207°. Yield 15 grams.

F. 2,7-Diaminofluorene - In a two liter flask was placed 50 grams of 2,7-dinitrofluorene, 800 ml. of alcohol, 250 ml. of concentrated hydrochloric acid, and 250 grams of granulated tin. The mixture was refluxed 2-3 hours, or until the solution becomes clear. The solution ~~is~~ was filtered and 500 ml. of the solution distilled. The residue was placed in a stoppered enlynmeyer flask and allowed to cool overnight, during which time a cream colored tin complex salt settled out. The mixture was filtered and the precipitate covered with 400 ml. of 3N sulfuric acid, stirred well, then filtered. This precipitate was again returned to a beaker, covered with 100 ml. of distilled water and 5 ml. of concentrated $NH_4OH$ added dropwise till just alkaline. Then 400 ml. of 3N sulfuric acid was added slowly, the solution stirred well, and filtered. The precipitate was washed twice with 50 ml. of distilled water. The sulfate salt was returned to a beaker, covered with 300 ml. of water, 100 ml. of concentrated ammonium hydroxide, and stirred mechanically for fifteen minutes. The mixture was filtered, the precipitate washed several times with water, then twice with a little alcohol and allowed to dry. Yield 34 grams. m.p. 163° - 164°.

G. 2,7-Diaminofluorene Dihydrochloride. - 2,7-diaminofluorene was dissolved in hot xylene and the solution filtered, using a hot water funnel. The solution was cooled and dry HCl gas passed into the xylene until no more material precipitated out. The white precipitate was filtered off, washed several times with ether, and allowed to dry. m.p. above 300°.

H. 2-Acetamido-7-Aminofluorene. - In a two liter 3-neck flask, equipped with a mechanical stirrer and a dropping funnel, was placed 35 grams (0.13 M) of 2,7-diaminofluorene dihydrochloride and 1100 ml. of water. The suspension was stirred rapidly and heated (60° - 70°) until all the precipitate was in solution. The clear solution was then cooled rapidly with an ice bath to room temperature. Then 13.00 ml. (0.13M) of acetic anhydride was added dropwise over a period of a half hour, with constant stirring. (After a few ml. of acetic anhydride had been added a precipitate formed.) After all the acetic anhydride has been added the mixture was stirred for an additional hour, then filtered and washed with a little water.

The precipitate was placed in a beaker, covered with 400 ml. of water and 75 ml. of concentrated ammonium hydroxide added slowly to the well stirred mixture. The precipitate was filtered and washed with water. Sintered 125°, m.p. 140°. After three recrystallizations from alcohol the precipitate melted at 198° - 199°. Yield 20 grams.

Anal. Calc. for $C_{15}H_{14}N_2OHCl$: N, 10.22%

Found: N, 10.32%

(The remaining 2,7-diaminofluorene may be recovered by adding 10 ml. of concentrated hydrochloric acid to the original reaction filtrate and refluxing the solution for twenty minutes. The solution was cooled and an excess of concentrated ammonium hydroxide added. The precipitate thus formed was almost pure 2,7-diaminofluorene.)

I. 2,7-Diacetamidofluorene. - In a one liter 3-neck flask, equipped with a mechanical stirrer, a reflux condenser and a dropping funnel, was placed 30 grams (0.15 M) of 2,7-diaminofluorene and 100 ml. of glacial acetic acid. The 2,7-diaminofluorene dissolved as the acetic acid was brought to reflux. Then 100 ml. of acetic anhydride was added, slowly at first. After all the acetic anhydride had been added the solution was refluxed for an additional two hours. Upon cooling it was poured into three liters of water and allowed to stand overnight. The next morning a tan precipitate was filtered and recrystallized from alcohol, using a few grams of Darco. Light yellow needles were obtained. m.p. 278° - 279°.

J. 2-Acetamido-7-N Methylaminofluorene. - To 30 grams (0.126 M) of 2-amino 7N-acetamidofluorene suspended in 1500 ml. of benzene (contained in a 2-liter 3-neck flask equipped with a mechanical stirrer, reflux condenser, and

dropping funnel) was added dropwise 45 grams (0.36 M) of dimethyl sulfate. After all the dimethyl sulfate had been added, the mixture was refluxed for 1.5 hours. A light gray precipitate was filtered which melted above 300°. This precipitate was covered with ammonium hydroxide solution and filtered. (The filtrate gave a positive test for sulfate ion by acidification, and addition of barium ion. The precipitate melting above 300° must have been the sulfate salt.) After recrystallization from acetone the material melted to a gel about 105° - 110°. By recrystallization from absolute alcohol the material sintered at 185°, m.p. 190° - 196°. Repeated recrystallization from absolute alcohol gave a constant melting point of 203° - 204°. A mixed melting point was taken with a pure sample of 2-amino-7-acetamidofluorene. The mixture sintered about 155° - 160°; melting at 180° - 185°.

Anal. calc. for $C_{16}H_{16}N_2O$: N, 11.12%

Found: N, 11.00%

K. 2-Acetylfluorene (48) - To 160 grams (0.96 M) of fluorene, in a 2-liter 3-neck flask (equipped with a mercury sealed mechanical stirrer and a reflux condenser connected to a hydrogen chloride trap) was added 900 ml. of dry carbon disulfide. After stirring the mixture a few minutes the fluorene dissolved. Then a half pound of aluminum chloride (226 grams, 1.70 M) was added and the mixture stirred thoroughly until it was homogenous. (A dark

red color developed.) Pure distilled acetic anhydride was added drop by drop (76 ml., 0.80 M). After the first ml. of acetic anhydride had been added, the reaction was initiated by warming the flask with the hands. The formation of a green color designated the initiation of the reaction. (If the reaction is not inhibited before the concentration of acetic anhydride becomes too great the reaction may become violent.) The further addition of acetic anhydride was then adjusted to such a rate that the heat of reaction keeps the carbon disulfide in gentle reflux. After all the acetic anhydride had been added the mixture was refluxed on a water bath ($45^{\circ}$ - $55^{\circ}$) for an additional hour.

The dark green mass was filtered with suction, and the precipitate sucked as dry as possible. The product was then placed in a 600 ml. beaker and just covered with carbon disulfide, stirred mechanically for ten minutes and filtered. The green precipitate was washed with two 50 ml. portions of carbon disulfide. After air drying for an hour, the precipitate was hydrolyzed by adding small portions of the precipitate to a mechanically stirred solution consisting of one liter of water and 60 ml. of concentrated hydrochloric acid. Yield 160 grams. m.p. $120^{\circ}$ - $127^{\circ}$.

Upon recrystallization from alcohol (20 grams of Darco, 110 grams of crude 2-acetylfluorene, and 800 ml. of alcohol) a white precipitate was obtained; m.p. $124^{\circ}$ - $126^{\circ}$. Further recrystallizations gave a constant melting point of

126° - 127°.

L. 4-(2-Fluoryl)- 2-Aminothiozole Hydroiodide. - Eighty grams (0.38 M) of 2-acetylfluorene (m.p. 126°), 60 grams (0.86 M) of thiourea, and 450 ml. of dioxane (peroxide free) were heated on a water bath and 100 grams (0.40 M) of iodine added over a period of 2.5 hours. Heating was continued for an additional 20 hours. On cooling and filtration an orange precipitate was recovered, which turned white upon washing with a little alcohol. Yield 100 grams (60% based on 2-acetylfluorene).

After recrystallization from 60% acetic acid the material sintered slightly at 290°, melting to a red liquid at 306°.

M. 4-(2-Fluoryl)- 2-Aminothiozole. - Twenty grams of 4-(2 fluoryl), 2-aminothioaole hydroiodide were covered with 60 ml. of 10% alcoholic ammonia. The mixture was mechanically stirred for a few minutes, filtered, then washed with a little alcohol.

The free amine was recrystallized from nitrobenzene. During the cooling of the nitrobenzene an atmosphere of nitrogen should be provided, otherwise oxidative impurities of indeterminate composition were formed. The free amine was separated from the mother liquor by filtration, washed with a little nitrobenzene, then washed three times with a little alcohol and dried in a dessicator under an atmosphere of nitrogen. m.p. 263°.

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

Found: N, 10.28%

N. 4-(2-Fluoryl)- 2-Acetamidothiazole. - Twenty-five grams of 4-(2-fluoryl), 2-aminothiazole hydroiodide were placed in a 500 ml. 3-neck flask. To it was added 250 ml. of alcoholic ammonia (10%), the mixture stirred for fifteen minutes, and then refluxed for a half hour. The suspension was poured into 1.5 liters of water. The cream colored precipitate was filtered and dried. Yield 20 grams.

The dry precipitate was covered with 100 ml. (1.0 M) of acetic anhydride, and the mixture slowly brought to reflux, while being mechanically stirred. The suspension was refluxed for a half hour and allowed to cool to room temperature. The cooled material was poured into 1.5 liters of cold water. After one hour all the excess acetic anhydride had reacted with the water. The suspension was allowed to stand for an additional hour, the precipitate filtered, washed with distilled water, then with a little acetone and allowed to dry. The compound sintered at 280°; m.p. 304° - 305°. After recrystallizing from acetophenone the material turned brown at 285°, melting at 304° - 305°.

Anal. calc. for $C_{18}H_{14}N_2OS$: N, 9.15%

Found: N, 8.91%

O. 2-Acetylfluorenone (49) - In a 5-liter flask, equipped with a reflux condenser and a dropping funnel, was added 2000 ml. of glacial acetic acid and 360 grams of

2-acetylfluorene. The mixture was heated until all the material had dissolved. Then 700 grams of sodium dichromate dihydrate was dissolved in a warm mixture consisting of 1300 ml. of glacial acetic acid and 375 ml. of water. The dichromate solution was added slowly at first (because of the violence of the initial reaction) and then more rapidly when the first violence had subsided. The solution was refluxed for two hours after all the dichromate solution had been added. The refluxing solution was then poured into 15 liters of water and allowed to stand overnight. The solution was filtered and the yellow precipitate was washed three times with 3N hydrochloric acid. Then the yellow precipitate was placed in a beaker, covered with 500 ml. of 4% potassium hydroxide, stirred mechanically for one hour and then filtered. After washing the precipitate twice with 150 ml. portions of 4% potassium hydroxide, it was washed several times with water. Sintered at 135°, m.p. 144°. Upon recrystallization from alcohol (650 ml./110 grams of ketone) using Darco, the yellow ketone melted at 153° - 154°. Yield 200 grams.

P. 4-(2-Fluoryl-9-one)-2-aminothiozole Hydroiodide. - To 130 ml. of dioxane (peroxide free) was added 25 grams (0.11 M) of 2-acetylfluorenone (m.p. 153° - 154°); 15 grams (0.23 M) of thiourea, and 28 grams (0.11 M) of iodine. The mixture was heated on an oil bath at c.a. 115°, so that the dioxane was in gentle reflux. The mixture was heated for six hours. Upon cooling, the solution was

filtered and an orange precipitate obtained, which turned bright yellow after washing with a little alcohol. Yield 25 grams. Sintered at 195°. m.p. ca. 303°.

Q. 4-(2-Fluoryl-9-one)-2-aminothiozole. - A water slurry of the yellow hydrochloride salt was treated with 6N sodium hydroxide until the solution was distinctly alkaline, and a red precipitate was formed. The precipitate was filtered and then dissolved in hot alcohol. The alcohol solution was filtered and three times the amount of water was added slowly with constant stirring. Upon filtration a red compound was obtained, sintering at about 190°, m.p. 195° - 200°. After recrystallizing from xylene (4 grams/100 ml. xylene) a material was obtained sintering at 194°, m.p. 198° - 201°. Further recrystallization did not alter the sintering or melting point.

Anal. calc. for $C_{16}H_{10}N_2O$: N, 10.07%

Found: N, 9.96%

R. 2-Chlorofluorene (50) - In a 2-liter 3-neck flask equipped with a mechanical stirrer was placed 330 grams (2.0 M) of fluorene and one liter of carbon tetrachloride. The mixture was cooled to 0° - 5° and 135 grams (1.93 M) of chlorine was added over a period of 3 - 4 hours with constant stirring. The carbon tetrachloride was distilled off on a water bath, and a waxy grey material remained. Yield 460 grams. m. p. c.a. 65°. One recrystallization from glacial

acetic acid raised the melting point to 86°. Further purification from glacial acetic acid and alcohol yielded a product melting at 95°.

S. 2-Chlor-7-nitrofluorene (51) - To 88 grams (0.44 M) of 2-chlorofluorene (m.p. 86°) was added 810 ml. of glacial acetic acid and the solution heated until the precipitate dissolved (50° - 60°). Then 120 ml. of concentrated nitric acid (sp. gr. 1.42) was added slowly to the well stirred solution.

If the temperature did not rise spontaneously, it was raised to 65° and allowed to rise spontaneously, while stirring vigorously. The temperature was not allowed to rise above 80°. A yellow precipitate formed and the solution turned to a paste. However, the paste was stirred vigorously to dissipate the heat, until the temperature reached approximately 60°. (When the temperature began to fall the dropping funnel was replaced by suction in order to remove the oxidizing gases.) The product was filtered on a Buchner funnel, sucked as dry as possible, then washed with two 50 ml. portions of glacial acetic acid, each containing one gram of potassium acetate, and then with one 50 ml. portion of glacial acetic acid. The 2-chlor-7-nitrofluorene was placed in a 2-liter beaker, covered with 1.5 liters of water and stirred mechanically for a few minutes and filtered. The last washing was repeated and the material air dried overnight. Yield 70 grams. The crude product sintered at 210°—

220° and melted between 222° - 235°. After two recrystallizations from glacial acetic acid the material melted at 243° - 244°. (Courtot - m.p. 237° from benzene)

T. 2-Chlor-7-aminofluorene. - In a liter round bottom flask, equipped with a reflux condenser, was placed 10 grams (0.04 M) of 2-chlor-7-nitrofluorene (m.p. 243°-244° ), 400 ml. of alcohol, 70 ml. of water, and 100 grams of zinc dust. The mixture was refluxed for two hours, filtered, and then poured into 3 liter of water. m.p. 136° - 138°. After recrystallization from 50% alcohol, with addition of Darco, the material melted sharply at 139° . Yield 7 grams (80%).

U. 2-Chlor-7-acetamidofluorene - To 7 grams (0.028 M) of 2-chlor-7-acetamidofluorene (m.p. 139°) was added 100 ml. of glacial acetic and 20 ml. (0.20 M) of acetic anhydride. The reaction mixture was slowly brought to reflux and allowed to reflux for 20 minutes. On cooling a white crystalline material settled out. Yield 7.6 grams, (91%), m.p. 228° - 230°. After three recrystallizations from glacial acetic acid, a constant melting point of 230° - 231° was obtained.

Anal. calc. for $C_{15}H_{12}ClNO$: N, 5.45%

Found: N, 5.23%

V. Fluorenone (52) - A 3-liter ring-necked flask was equipped with an addition tube carrying a 500 ml.

dropping funnel and a water condenser. In the flask were mixed 200 grams (1.2 moles) of technical fluorene and 400 ml. of technical glacial acetic acid and the mixture heated to gentle boiling. In another flask 600 grams of technical crystals of sodium bichromate were dissolved in a warm mixture of 800 ml. of glacial acetic acid and 200 ml. of water. By means of the dropping funnel this warm oxidizing solution was added in a slow steady stream to the boiling fluorene solution, so that the boiling was never interrupted and no precipitation of lumps occurred. This required about half an hour, as at first the reaction was quite vigorous. The mixed solutions were then refluxed for two and one-half hours more. The deep green liquid was then poured into 4 liters of ice water, allowed to stand for at least two hours and finally filtered through cloth on a 120 mm. Buchner funnel. The first wash water contained a little sulfuric acid to avoid possible hydrolysis of the chromium salts, but the residue on the filter was washed free from all trace of chromic ion with pure water. The bright yellow residue weighed 190-200 grams.

The air-dried crude fluorenone was placed in a 500 ml. Claisen distilling flask arranged to distil into an ordinary 300 ml. distilling flask, the Claisen side-arm serving as the only condenser. The Claisen flask was provided with theusual tube for the admission of air in vacuum distillations. (The upper part of the Claisen may advantageously be wound with asbestos paper or twine.) The flask was preferably

heated with an electric cone heater as this facilitates smooth distillation. The receiver was connected to a vacuum pump (suitably protected from vapors and preferably arranged with pressure regulator) or water pump and the fluorenone distilled under reduced pressure. Toward the end of the distillation the clear bright yellow liquid acquired an orange tinge, the appearance of which serves as a convenient signal to stop the distillation. The temperature range of distillation was not particularly important because its purpose was merely to remove rubicene and other highly colored impurities which cannot be separated by crystallization.

The clear bright yellow distillate was dissolved in that number of ml. of benzene numerically equal to its weight in grams, this solution warmed and to it slowly added twice the benzene volume of petroleum ether, b.p. 30-60°, in such a way that no permanent separation of precipitate occurred. On allowing this solution to stand, extraordinarily beautiful crystals of fluorenone separate. These were filtered off, washed with petroleum ether and dried in the air. The yield based on the original crude fluorenone was 60-70% of crystals melting at 83.0-83.5°, uncorr.

W. 9-Fluorenoneoxime (53) - In a 1-liter flask equipped with a reflux condenser was placed 40 grams (0.22 M) of fluorenone (m.p. 83°), 600 ml. of ethyl alcohol, 23.0 grams (0.33 M) of hydroxylamine hydrochloride, and 35.6 grams

(0.18 M) of barium carbonate. The mixture was heated on a hot water bath for three hours, then filtered through a hot water funnel. On cooling yellow crystals settled out. Concentration of the mother liquor gave a second crop of crystals. Yield 41 grams. m.p. 192° - 193°.

X. 9-Aminofluorene (54) - In a one liter three-neck flask equipped with a reflux condenser and a mechanical stirrer was placed forty grams of fluorenoneoxime, 270 ml. of glacial acetic acid and 13 ml. of water. The solution was brought almost to reflux and 70 grams of zinc dust was added at such a rate as to maintain reflux. After all the zinc dust was added the mixture was heated for an additional hour. Then 400 ml. of water was added and the solution filtered. The residue was extracted with 200 ml. of hot 5% acetic acid. The combined acetic acid solutions were mixed with 850 ml. of concentrated hydrochloric acid. The mixture was kept at 0° for 10 hours. The 9-aminofluorene hydrochloric was filtered and washed with 35 ml. of cold concentrated hydrochloric acid. The hydrochloride was recrystallized from boiling water.

The hydrochloride was reacted with 100 ml. of solution containing 40 ml. of concentrated ammonium hydroxide, filtered, and dried over calcium oxide. m.p. 64-65°. Yield 30 grams.

Y. 9-Acetamidofluorene. - To a slowly refluxing

solution (mechanically stirred) consistingof 350 ml. of glacial acetic acid and 40 grams (0.20 M) of 9-fluorenonoxime was added 50 grams (0.77 M) of zinc dust in small increments, over a period of a half hour. After refluxing the mixture for an additional hour, 55 ml. (0.55 M) of acetic anhydride were added dropwise to the refluxing solution. When all the acetic anhydride had been added, the solution was refluxed for an additional hour. The mixture was filtered through a hot water funnel and allowed to cool. A white precipitate settled out. The precipitate was filtered and washed with a little acetic acid. Yield 45 grams. m.p. 246°. Subsequent recrystallization from acetic acid did not alter the melting point.

Anal. calc. for $C_{15}H_{13}NO$: N, 6.28%

Found: N, 6.17%

## SUMMARY AND CONCLUSIONS

The biological experimental work of this project is being carried out at the Memorial Hospital in New York City. Due to difficulties encountered there in the breeding of mice suitable for the tests and the disruption of their laboratories because of an expansion program undertaken by the hospital, only fragmentary evidence has as yet emerged. Therefore, the report of the results must wait until all evidence has been collected in order to evaluate properly the scope of the work.

During the course of this work the following compounds have been described for the first time.

N, N, B-dichloroethyl-2-aminofluorene

2-acetamido-7,N-methylaminofluorene

4-(2-fluoryl)-2-aminothiazole hydroiodide

4-(2-fluoryl)-2-aminothiazole

4-(2-fluoryl)-2-acetamidothiazole

4-(2-fluoryl-9-one)-2-aminothiazole hydroiodide

4-(2-fluoryl-9-one)-2-aminothiazole

2-chlor-7-acetamidofluorene

The syntheses of the following compounds have been improved in either yield or ease of production or both over those methods now existing in the literature.

2-nitrofluorene

2,7-diaminofluorene

2-acetamido-7-aminofluorene

2-chlor-7-nitrofluorene

2-chlor-7-aminofluorene

9-acetamidofluorene

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