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I hereby recommend that the thesis prepared under my
and that of Dr. F. E. Ray
supervision, by Richard J. Garascia
entitled Fluorene Arsenicals

be accepted as fulfilling this part of the requirements for the
degree of Doctor of Philosophy

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FLUORENE ARSENICALS

A dissertation submitted to the
Graduate School of Arts and Sciences
of the University of Cincinnati
in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

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ACKNOWLEDGEMENT

The author acknowledges with pleasure the patient direction and gracious encouragement of Dr. Francis E. Ray and, in the later stages of the research, the helpful suggestions of Dr. E. F. Farnau.

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FLUORENE ARSENICALS

1. INTRODUCTION

Organic arsenicals have, since the days of Ehrlich and his monumental researches, been the subject of ever increasing investigation. Many thousands of compounds have been prepared; a good number of these have been tested for therapeutic value against, for example, trypanosome infection and the syphilis spirochete; and several more or less trustworthy rules of thumb have patiently been constructed to correlate, to a minor extent, chemical constitution of arsenicals with their therapeutic value.

In the search for better arsenicals, the polynuclear and heterocyclic systems have been, to some degree, neglected in favor of the benzene derivatives. This is probably a reasonable negligence, since the greater facility in preparation of benzene compounds, their higher solubilities, and the more easy assimilation of these by the body have been three factors of merit in their favor. Yet, in the light of how little is actually known of the mechanism of arsenical action, evidently no aromatic or heterocyclic system should be ignored, and useful compounds will undoubtedly be found, if they do not already exist, for each of these parent

systems. Thus, increasing investigation of the arsenicals of naphthalene, phenanthrene, anthracene, and the like, is to be expected.

Now fluorene arsenicals have been the subject of four chemical papers published more than a dozen years ago. All of these compounds were based on the parent fluorene-2-arsonic acid. In several cases, the compounds showed favorable, but not remarkable, pharmacological properties. In view of the many possibilities offered by this hydrocarbon, it is the object of this research to extend the number of fluorene arsenicals to include new types. Our object is two-fold: to prepare new arsenicals for medical application, and, secondly, to extend, in so doing, the chemistry of the fluorene system.

II. ORGANIC ARSENICALS. GENERAL DISCUSSION

A. Nomenclature.

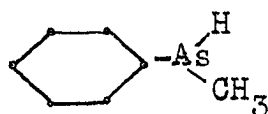
The naming and indexing of organic arsenic compounds presents, even at present, a few difficulties not entirely surmounted. No wide scale adoption of any particular system has been made, and there is as yet an appreciable variation between naming systems used in this country and those used in England. The nomenclature adopted here is essentially that proposed by the International Union of Chemistry in 1936 and 1938, and pretty well adopted by CHEMICAL ABSTRACTS since 1937 (20).

TABLE 1

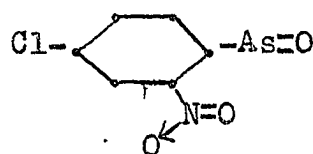
<u>Radical</u>	<u>Prefix</u>	<u>Suffix</u>
-AsH ₂	Arsino	Arsine
-AsO	Arsenoso	----
-AsO ₂	Arso	----
=As(O)OH	Arsinico	Arsinic acid
-As(O)(OH) ₂	Arsono	Arsonic acid
-As=As-	Arseno	----
-As(OH) ₂	----	Arsonous acid
=AsOH	----	Arsinous acid
-p=As-	Phospharseno	----
-Sb=As	Stibarseno	----

Table I above lists radicals together with the proper literal use of prefix and suffix. All other compounds not namable according to the list given should be named, if possible, as substituted arsines.

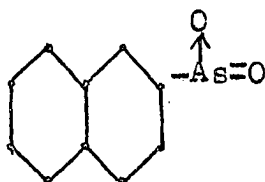
Examples of the nomenclature follow. The usual convention employing the dash (-) to indicate covalent bonds and an arrow ($\rightarrow$) to designate coordinate bonds between donor and acceptor atoms is retained.



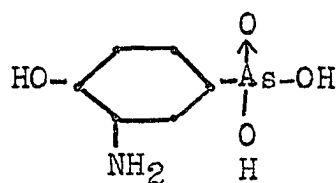
methylphenyl arsine



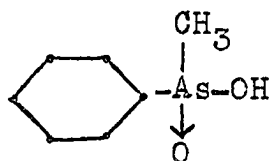
2-nitro-4-chloro-arsenosobenzene



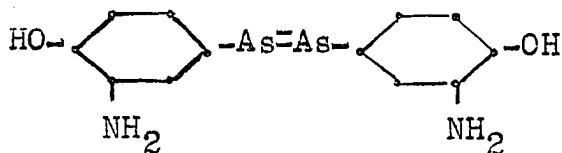
2-arsonaphthalene



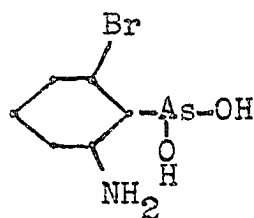
3-amino-4-hydroxyphenyl arsonic acid



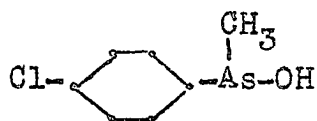
methylphenyl arsenic acid



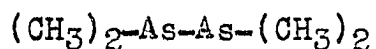
3,3'-diamino-4,4'-dihydroxy arsenobenzene



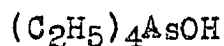
2-bromo-6-amino arsenous acid



methyl-p-chlorophenyl arsinous acid



tetramethyldiarsine



tetraethylarsonium hydroxide

In the following sections, all chemical names will follow the above rules except where, in cases of the very important and well-known arsenicals, common names have largely been adopted.

B. The Theory of Therapeutic Activity.

Although much used in near-ancient days for treatment of various diseases, inorganic arsenic compounds are now but seldom employed as pharmaceutical reagents. The high toxicity of the compounds, especially with soluble trivalent forms, makes application extremely dangerous. The most important inorganic preparation still in use is "Fowler's solution", a dilute water solution of potassium arsenite. It is employed as a tonic and for partial relief in cases of anemia, Hodgkin's disease, and rheumatism. It is also used externally for treatment of skin disorders such as psoriasis and chronic eczema. The preparation appears in the U. S. Pharmacopeia (88).

Indeed, the first general symptom of arsenic poisoning is one of increased appetite and body weight. The skin takes on a healthier appearance and the red-cell count of the blood increases. The use of white arsenic (As_2O_3) taken internally by certain natives of Styria and Tyrol is said to increase general bodily health and resistance to physical fatigue. The extravagant claims made by these "arsenic eaters" are not substantiated in fact, though apparently, harmful effects need not follow since many of the users are long-lived (29).

The use of aliphatic organic forms is limited to a few preparations. Here, again, the toxicity is high, probably because of easy hydrolysis in the body to inorganic arsenic.

The compounds still occasionally applied (73) are given in Table II.

TABLE II

<u>Compound</u>	<u>Formula</u>	<u>Application</u>
sodium cacodylate	$(\text{CH}_3)_2 = \text{As} \begin{array}{l} \nearrow \text{O} \\ \searrow \text{ONa} \end{array}$	psoriasis and other skin dis- eases; anemias, leukemia (oral or external).
Solarson	$\text{CH}_3(\text{CH}_2)_4\text{CCL} = \text{CH} \cdot \text{As} \cdot \text{O}(\text{OH})\text{ONH}_4$	anemias, (oral, subcut., or in- tramuse.)
guaiacol cacodylate	$(\text{CH}_3)_2\text{AsO} \cdot \text{O} \cdot \text{C}_6\text{H}_4\text{OCH}_3, \text{H}_2\text{O}$	regional tuber- culosis (oral or subcut.)
Elarson	strontium chloroarseno behenate (uncertain struc- ture)	exophthalmic goiter, anemias, neuralgia (oral).

Aromatic arsenicals have proved more advantageous in the treatment of protozoal and bacterial infections. The first preparation of an aromatic compound was accomplished by Bechamp (8), in 1860 by heating aniline arsenate to 190-200°C. The structure of the compound was correctly established by Ehrlich and Bertheim (45) who showed it to be p-aminophenyl arsonic acid.

Beginning in 1875, Michaelis (76) and co-workers prepared many aromatic arsenic compounds and extended the methods for preparing them to include entirely new procedures. The

pharmaceutical importance of the compounds was recognized by Ehrlich when it was shown by Thomas (14), and Breinl and Kinghorn (16) that the p-aminophenyl arsonic acid of Bechamp, popularly called atoxyl or arsanilic acid, had therapeutic effects on experimental trypanosomiasis.

After many years of investigation, during which Ehrlich and his students developed new arsenicals which they tested for trypanocidal activity, the compound referred to variously as arsphenamine, salvarsan, kharsivan, or "606" was prepared (47) which showed remarkable trypanocidal properties as well as good action against spirillae and spirochetes. The use of the compound against syphilis, framboesia, recurrent fever, etc., continues to the present day. Further investigation of aromatic arsenicals by many research workers has resulted in the preparation of many thousands of compounds, some of which have proved valuable in chemotherapy. A list of the more important of these is given in Table III.

Some serious effects follow the use of certain arsenicals, or the too frequently repeated use of others. Besides general symptoms of chronic arsenic poisoning, delayed action on the nervous system sometimes appears. Thus, optic nerve injury, general neuritis, and mental deterioration may result (60). This is particularly so with atoxyl, arsacetin and tryparsamide. It is known, too, that parasites may become immune to an administered preparation in time and

TABLE III
AROMATIC ARSENICALS IN USE

<u>Compound</u>	<u>Structure</u>	<u>Use</u>	<u>Dosage</u>
Arsphenamine		syphilis, relapsing fever, frombesia, malaria, Hodgkin's disease.	0.3-0.4 g. (intraven.)
Neoarsphenamine		Primary syphilis	0.6 g. (intrav.)
Sulfarsphenamine		spirillicide, general syphilis.	0.400.5 g. (intraven. intramusc.)
Silver arsphenamine		like Arsphenamine	0.1-0.3 g. (intraven.)

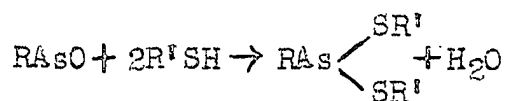
TABLE III (Cont'd.)

<u>Compound</u>	<u>Structure</u>	<u>Use</u>	<u>Dosage</u>
Acetarsonsone	$\begin{array}{c} \text{HO}-\text{C}_6\text{H}_4-\text{AsO}_3\text{H}_2 \\ \\ \text{CH}_3\text{CONH} \end{array}$	amebiasis, congenital syphilis, Vincent's angina.	0.25 g. (oral)
Mapharsen	$\begin{array}{c} \text{HO}-\text{C}_6\text{H}_4-\text{As}=\text{O} \\ \\ \text{HCl} \cdot \text{NH}_2 \end{array}$	syphilis	0.03-0.04 g. (intraven.)
Tryparsamide	$\text{NH}_2\text{COCH}_2\text{NH}-\text{C}_6\text{H}_4-\text{AsO}_3\text{HNa}$	trypanocide, neuro-syphilis, early paresis	1-3 g. (intraven.)
Carbarsone	$\text{NH}_2\text{CONH}-\text{C}_6\text{H}_4-\text{AsO}_3\text{HNa}$	intestinal amebiasis.	0.25 g. (oral)
Arsacetin	$\text{CH}_3\text{CONH}-\text{C}_6\text{H}_4-\text{AsO}_3\text{HNa}$	antisiphylitic, protozoal diseases.	0.5-0.6 g. (intraven.)

resistant strains of the bacteria or protozoa appear (36). Usually, the resistance is specific to one compound, and a change in the compound administered is then indicated. The compound (p-arsenosophenyl)-butyric acid has recently been described as particularly effective against trypanosomes since it does not readily give rise to resistant strains (37).

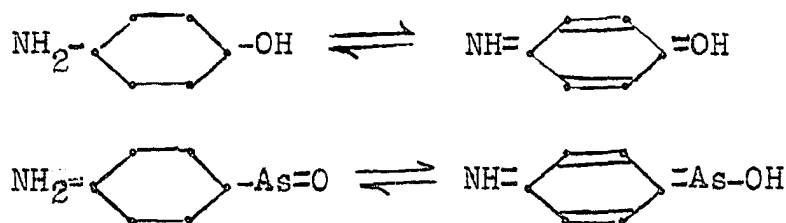
Now the theory of therapeutic action of these compounds includes the belief that the trivalent forms are the most potent, and that other forms (arseno compounds and arsonic acids) are first changed in the organism to the arsenoso compounds which represent the effective type. This belief is substantiated by the fact that many arsonic acids (for example, atoxyl or arsacetin) are inactive in vitro but active in vivo (44). The compound 4-hydroxyphenyl arsonic acid will not kill trypanosomes in a test tube in dilutions of 1 to 2% but the reduced form 4-hydroxy-arsenosobenzene will completely sterilize trypanosomes in vitro at a concentration of 1:10,000,000. Furthermore, a parasite made drug-fast (immune) to a trivalent compound will exhibit, likewise, immunity toward the corresponding pentavalent compound and vice versa. (92)

The presence of the arsenoso group is said to disturb the oxidation-reduction mechanism in the tissues of the parasite, and to a lesser extent of the host. Such action probably involves the sulfhydryl groups present in the protein structure (101, 117).



It is well known that certain sulfhydryl compounds like cysteine, thioglycollic acid and thiolactic acid have inhibiting effects in the administration of arsenoso compounds. Action of the arsphenamines is also delayed (31,32,33,118).

Reiner and Leonard (94) suggested a formation of loose compounds of arsphenamine with serum proteins. Such loose addition compounds can sometimes be formed in the test tube. In the case of a series of substituted arsenosobenzene compounds, it was found (64) that the systemic toxicity of these could be directly related to the extent of binding by red blood cells either in vivo or in vitro. Also postulated (95) is the formation in the body of compounds of the quinone imine type.



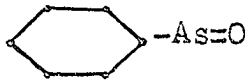
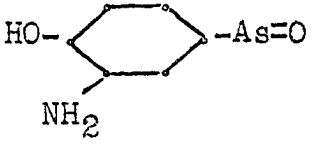
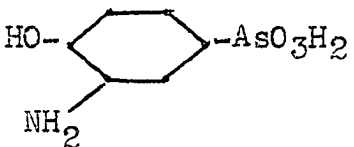
The oxidation-reduction concept of arsenical action seems, then, well established and is in accord with the theory of Bing and Sculy (29) in regard to inorganic arsenical action in which alternate change from penta-valent to trivalent arsenic is said to be the main action in withdrawing oxygen from or giving oxygen to the living tissues.

Now aside from the main action of the arsenic group in organic arsenicals, it was very early recognized that the organic residue has much to do with the variation in toxic and curative properties. Ehrlich (41, 42, 43, 44) developed some clear notions concerning the mechanism of drug action. According to his views, an effective compound is at once possessed of a group poisonous to the parasite (toxophore) and a group capable of anchoring itself to the cell walls of the parasite (haptophore). It is to this latter kind of group that the specificity and special action of the compound is due. In the case of the benzene arsenicals, the therapeutic results vary with the kind, number, and relative positions of the groups. Ehrlich found that the introduction of a para salt-forming group ($-\text{OH}$, $-\text{NH}_2$) was generally helpful in yielding desirable properties, and that a third substituent group ($-\text{Cl}$, $-\text{NH}_2$, $-\text{OH}$) ortho to the $-\text{OH}$ or $-\text{NH}_2$ usually increased the favorable effects. An ortho $-\text{CH}_3$ or $-\text{NO}_2$ group, however, in p-arsanilic acid increases the toxic qualities.

To illustrate these general principals, comparison information is presented in Table IV. The organisms most frequently used for testing are trypanosoma equiperdum, trypanosoma rhodisiense and trypanosoma lewise. Therapeutic index is defined as the ratio of minimum lethal dose (minimum weight of drug per unit body weight capable of killing the host) to minimum curative dose (minimum weight

TABLE IV

(Therapeutic values in milligrams per 20 grams of mouse)

<u>COMPOUND</u>	<u>STRUCTURE</u>	<u>M.L.D.</u>	<u>M.C.D.</u>	<u>T.I.</u>
Arsenosobenzene		0.05	0.025	2
p-hydroxyarsenosobenzene		0.20	0.038	5.3
p-aminoarsenosobenzene		0.10	0.035	2.8
Mapharsen		0.45	0.048	9.4
Atoxyl		7.1	4.8	1.5
Tryparsamide		40.0	4.5	9
Carbarsone		4.0	1.0	4
Arsacetin		40.0	28.0	1.4
p-hydroxy-m-amino-phenylarsonic acid		12.6	7.6	1.6

of drug per unit body weight capable of killing the parasite.) These three terms are frequently abbreviated to T.I., M.L.D., M.C.D.

The data of Table IV should be accepted with some caution since the literature is not always consistent as to therapeutic values. Results depend on methods of administration, the parasite employed, and the kind of animal submitted to the test. A real service to chemotherapy could be done by a systematic standardization of values of the many compounds so far tested. Only then can sure interpretation of the correlation between structure and therapeutic effects be made.

In general, arsenoso compounds are more toxic than arsonic acids, whereas arseno derivatives are least toxic of all. Trivalent arsenicals seem to be useful generally as treponemicidal agents (for example, against the syphilis spirochete) whereas the arsonic acids are more effective against protozoal disorders. Tryparsamide is effective in late syphilis, but not in the early stages.

The introduction into the benzene nucleus of a para salt-forming group ($-\text{OH}$, $-\text{NH}_2$) lowers the toxicity as well as the activity of the compound. Sometimes, more favorable therapeutic ratios result. The methylation of the phenolic or amino group is usually unsatisfactory, but acetylation, particularly of the amino group, is very effective for further reducing toxic qualities. Nitro

groups are usually without effect, and halogen atoms often enhance by a good deal the toxic nature of the compounds. The therapeutic index or ratio is a more important criterion than toxic or curative doses considered in themselves. Mapharsen is much more toxic than arsphenamine, but the curative properties of mapharsen are simultaneously superior, and the therapeutic index of the compound is of an order that makes it a valuable drug.

Eagle (35, 38, 39, 40) has made an extensive study of the effect of substituent groups on the therapeutic properties of arsenosobenzene (arsenoxide) and some general principles relating to this series have been advanced.

The goal is, of course, to synthesize compounds with a high (safe) therapeutic index and of low toxicity. The arsphenamines are still the best arsenicals from this standpoint, but suffer from ease of oxidation and lack of purity.

The fate of the organic arsenical in the body has been the subject of scant investigation. R. R. Williams (120) has made an effort to collect some of the information. Apparently, a large quantity of the organic arsenical can appear unchanged in the urine. For example, cacodylic acid is excreted as such to the extent of 14% (62). Some amount of unchanged arsphenamine can likewise be detected. In the few cases where changed metabolites have been identified, there is evidence that the mechanism of detoxica-

tion is no simple one. In the case of arspenamine, compounds isolated in the excreta were p-aminophenol, o-acetaminophenyl sulfate, benzoxazolone, 3-hydroxy-4-amino-phenyl arsonic acid, and p-hydroxyphenyl arsonic acid (110). Inorganic arsenic is also a common form for excretion (85).

C. Clinical Methods of Evaluation.

The clinical or experimental methods of assay or evaluation of drugs is, generally, a laborious and painstaking procedure. The difficulties arising from proper control and standardization of conditions, the selection of experimental animals conforming to definite standards, duplication of proper strains of parasites used for inoculation, and the proper interpretation of results gathered, sometimes over periods of many months, present difficulties not entirely surmountable. As a rule, evaluations made must be re-checked for proper accuracy and interpretations of the data made with full knowledge of the failings of the methods involved. Usually, results on a series of compounds cannot be properly correlated unless determined under identical conditions, and usually in the same clinic or laboratory by the same workers.

The various therapeutic values will differ, of course, not only with regard to the parasite chosen for testing, but also with the type of experimental animal. Evaluations may be done in vivo when the various curative and lethal doses may be determined on rats, rabbits

and the like; or they may be done in vitro when the effect of drugs on parasite cultures in the test tube is observed.

In the case of arsenicals, the parasite chosen is one against which the arsenical type has been found more or less effective. Hence, the choice may be the syphilis spirochete since treponemicidal activity is frequently present; or may be one of many protozoal forms. Arsenicals are effective against amoebic dysentery caused by E. histolytica, and against various forms of trypanosomiasis: African sleeping sickness caused by T. rhodesiense, African trypanosomiasis caused by T. gambiense, and South American Chaga's disease caused by T. cruzi. A popularly used trypanosome injection is the trypanosomiasis in rats caused by T. equiperdum. This last parasite is easy to keep alive and responds well to clinical methods. It has been shown (105, 118) that there is a close correlation between therapeutic effects of arsenicals on rats infected with T. equiperdum and those observed clinically with treponema pallidum (syphilis spirochete). Other artificially induced diseases used in standardization are: spirillosis in hens or rabbits, recurrent fever in mice, syphilis in rabbits.

In the case of in vivo assaying for curative properties, a carefully chosen and standardized strain of

parasite is inoculated into a seed animal (usually rabbit) and a period of time between 24 and 48 hours allowed to elapse for proper multiplication of the organism. At the end of this time, a sample of blood from the seed animal is checked and a count of parasite bodies per ml. is made. Certain limits are acceptable, for example 30,000-50,000 or 150,000-250,000 parasites per ml. of blood depending on the particular disease. The seed animal is then killed and the extracted blood, in measured quantities, is injected into a group of experimental animals. Generally, a group of 5 to 10 animals of carefully controlled age, weight limits, and diet are required for each dosage of the drug to be administered. After 24 to 48 hours, a count is again made on each of the animals and a carefully measured amount of drug is administered, usually intravenously. All factors, such as concentration and rate of injection must be standardized. The amount of drug is calculated in terms of weight of drug per weight of animal body. Blood counts are made periodically, say at the end of 12, 24, 48, 72 hours and the various effects are noted. The animal is judged cured if the parasite count falls below a certain minimum number and remains such.

Many variations of the methods indicated above exist.

In the older procedures, animals were infected with virulent strains known to produce death in the short time of one to several days, and efficacy of the drug was gauged by the number of experimental animals remaining alive at the end of a fairly long interval (91). In other non-counting methods, the animal is watched, after administration of the parasite, for well developed symptoms of the disease (several days to several weeks), then is given a measured dosage of the drug and the effects observed. Useful procedures have been formulated by the U. S. Public Health Service and other governmental laboratories (34,83).

The toxicity of the compound is determined in vivo by administration of increasing doses of the drug to experimental animals (4-6 for each dose) and the number of deaths in a specified interval of time noted (34). A method has been suggested wherein the time elapsed before death is taken as a graded response method for toxicity determination (87).

In vitro methods for estimation of curative properties offer decided possibilities. These tests are, of course, very easy to apply and take only a few minutes to several hours for determination. The method usually consists in the addition of a definite amount of compound to a culture of the appropriate organism, and the effect is

followed by microscopic count at frequent intervals over a fairly short time period. Such in vitro tests cannot be used with arsphenamine types or pentavalent arsenicals which depend for their curative effect on certain redox changes while in the animal host. The method has been advocated (21,34) for assay of the arsenoso compounds (arsenoxides).

In describing results of such clinical evaluation, certain terms have become common. The dosage (calculated as weight of drug per weight of animal) averagely just sufficient to cure the diseased animal is called the minimum curative dose (M.C.D.). The dosage averagely just sufficient to kill the experimental animal is the minimum lethal dose (M.L.D.). Other dosages are important besides: that which is averagely just sufficient to have a minimal measureable effect on the parasite is called the minimum therapeutic dose, while the dosage above which more than a low percentage (say 5%) of the animals would die is termed the maximum tolerated dose. The proper mathematical interpretation of the results has been investigated (15).

The therapeutic value or index usually refers to the ratio of minimum lethal dose (M.L.D.) to minimum curative dose (M.C.D.).

It is to be remarked that results obtained by assay with experimental animals are not necessarily indicative

of corresponding efficacy when used on humans, even for the same diseases. Cautious clinical evaluation with humans must always follow the preliminary operations. Yet, usually, these first therapeutic studies are relatively in proper order and serve to point out which of the drugs possess promising characteristics and which are useless.

D. Aromatic Arsenicals.

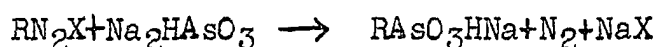
Since therapeutically valuable compounds are almost exclusively arsonic acids (RAsO_3H_2), arseno ($\text{RAs} = \text{AsR}$), or arsenoso compounds (RAsO), general methods for the preparation of these will be given.

1. The Arsonic Acids (RAsO_3H_2).

The Bechamp Reaction. Historically the first direct method to be discovered (8), the reaction consists in heating an aromatic amine or phenol with arsenic acid at somewhat elevated temperatures (150-200 C.) for periods ranging from several hours to several days. An amino or phenolic arsonic acid is produced. Direction is ortho and para in all such cases. The para position is most readily attacked; ortho substitution ordinarily takes place only if the para position is blocked, and, in such cases, the yields are usually poor. No solvent is employed and efficient stirring is frequently the critical feature of the reaction. The arsenic acid used varies

from a highly concentrated aqueous solution (80-85%) to solid anhydrous arsenic acid. Most frequently, in arsonation of amines, the amine is in appreciable excess (2-3 moles) and acts somewhat as the solvent. Arsonation of phenols generally involves an excess of arsenic acid. Yields range from 1% to 60%, with an average yield of about 20%. The reaction probably takes place through the formation of the arsenate and arsamie acid very much like the bake process for sulfanilic acid. Some small quantities of the arsinic acid (R_2AsO_2H) can sometimes be isolated. Preparation of arsanilic acid by this method and preparation of p-hydroxyphenyl arsonic acid are described in detail in Organic Syntheses (56).

The Bart Reaction. This more generally used and more successfully applied reaction represents the best method for the preparation of arsonic acids (7). An aromatic amine is diazotized in the usual way and the diazonium solution is decomposed in the presence of aqueous arsenite and a small amount of catalyst.

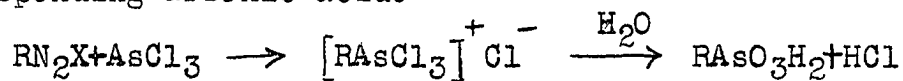


Sodium or potassium arsenite, or the equivalent solution of arsenious oxide in sodium or potassium hydroxide or carbonate, may be used. Buffers, such as sodium carbonate, bicarbonate or acetate, are frequently employed.

The yield is apparently dependent on careful control of pH

which should be constant throughout the procedure and somewhat on the basic side. The catalyst used is frequently copper sulfate, but may represent other cupric or cuprous salts, metallic copper, nickel or cobalt, or salts of nickel and cobalt. Sometimes, a second reducing agent is simultaneously employed (sodium hydrosulfite, hypophosphorous acid, sodium formaldehyde sulfoxylate). Yields vary with the ease of diazotization of the original amines. Average yield is about 50%, but is frequently much better. Diazonium fluoborates have been newly employed in the Bart method with occasional improvement of yield.(102).

The Scheller Reaction. Really a modification of the Bart procedure, this reaction used by Scheller to good advantage (106), employs a non-aqueous medium of alcohol (methyl or ethyl) or glacial acetic acid for diazotization, followed by decomposition in the presence of anhydrous arsenic trichloride, with a trace of catalyst, commonly cuprous halides. The arsenic trichloride is sometimes added before diazotization. Removal of the solvent by distillation followed by hydrolysis gives the corresponding arsonic acid.



This method frequently gives good yields of products where the Bart reaction fails, but it is not always better than the Bart. Weak amines (for example,

p-nitroaniline) frequently yield better results with the Scheller reaction. We have found that acetone can be used successfully as solvent for amines of the fluorenone series.

The Bechamp, Bart and Scheller reactions are treated in the series, "Organic Reactions" (1).

Miscellaneous Methods. The method of Rosenmund (100) is applicable only to a few compounds. An active aromatic halogen compound is heated with potassium or sodium arsenite in boiling alcohol-water solution. Sealed tube techniques are sometimes necessary. Copper catalysts have been used. Yields are as high as 45%. This method is an extension of the more general Meyer reaction in the aliphatic field (75).

Oxidation of trivalent arsenic types can lead to arsonic acids. Thus aromatic arsines (RAsH_2) may be oxidized to arsonic acids by nitric acid; arsenoso compounds (RAsO) may be oxidized with hydrogen peroxide; and arseno (RAs=AsR) types may be oxidized with iodine. Aromatic dichlorarsines (RAsCl_2) may be converted to the tetrachlorides with chlorine gas, then hydrolyzed to give arsonic acids. Since these trivalent compounds are frequently synthesized from arsonic acids, these methods are decidedly limited in application.

Properties of the arsonic acids. The arsonic acids, as a class, are well-defined. Some may be crystallized

from water, but more often they are water insoluble. They may be purified through their sodium, potassium or ammonium salts which are, as a rule, easily soluble in water. As dibasic acids, they can form two series of salts in many cases. The heavy metal salts are frequently insoluble in water. Arsonic acids give no precipitates with cold magnesia mixture, but an insoluble salt usually forms on heating, and this fact has been employed in the separation of arsonic acids from inorganic arsenic residues.

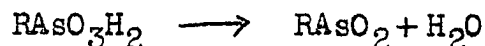
Some few arsonic acids have been investigated as qualitative or quantitative precipitating agents (54). The arsonic acids seem to combine with and precipitate the quadrivalent metallic elements of the fourth periodic group. Both aliphatic and aromatic arsonic acids have been investigated. The most widely used are phenyl arsonic, n-propyl arsonic, p-hydroxyphenyl arsonic, and p-n-butylphenyl arsonic acids. Thus, zirconium alone, or in the presence of aluminum, beryllium, bismuth, copper, iron, manganese, nickel, trivalent earths, and zinc has been estimated with phenyl arsonic acid (96). Zirconium has also been quantitatively determined with n-propyl arsonic acid (3), and with p-hydroxyphenyl arsonic acid (111). Other applications have been made to thorium, titanium, tin, and bismuth. The precipitated salt is not ordinarily weighed as such but ignited

in air or a current of hydrogen to the oxide of the heavy metal.

The arsonic acid group is ordinarily firmly attached to the nucleus, permitting nitration and halogenation of the ring and oxidation of side-chains. Nitro groups may be reduced to amines (catalytically, or with ferrous compounds, sodium amalgam, or cold hyposulfite) keeping the arsonic acid group intact, and the resulting amino group may be diazotized and replaced in the usual manner.

The compounds are fairly strong acids. Ionization constants of some of them have been determined (89). Primary ionization constants are of the order 1×10^{-4} and secondary constants of the order 1×10^{-9} . Electrical conductivities have been investigated in the hope of finding some correlation with pharmacological attributes (104).

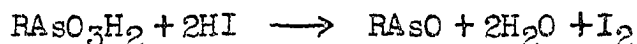
Some arsonic acids have good melting points, but the majority of them form anhydrides or decompose in the course of heating.



2. Arsenoso Compounds (Arsineoxides) (RAsO).

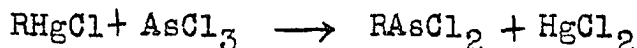
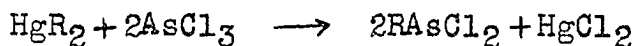
These compounds are most readily prepared directly or indirectly from the corresponding arsonic acids. Arsonic acids can be directly reduced to the arsenoso form by treatment with sulfur dioxide in the presence of a trace of hydriodic acid. Sulfurous acid alone is

slow to act, but the hydrogen iodide is said to act as an intermediate catalytic reagent.



The iodine is then reduced to HI by the action of sulfur dioxide. Other reducing agents such as phenylhydrazine or phosphorous acid, in alcohol or ether medium, have been employed.

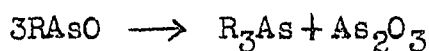
Treatment of arsonic acids with phosphorus trichloride in inert solvents, or with sulfur dioxide in an excess of hydrochloric acid, transforms them into dichlorarsines (RAsCl_2). Hydrolysis of these compounds with dilute aqueous alkali or carbonate gives the desired arsenoso derivatives. Halogen acids reconvert the compounds to the corresponding dihaloarsines. Similarly, reduction of the arsonic acid group with hydriodic acid gives the di-iodoarsine (RAsI_2) which can then be hydrolysed to RAsO . Compounds of the type RAsCl_2 can also be made from mercury diaryls or aryl mercurichlorides by treatment with arsenic trichloride.



Hydrolysis, again, is the final step to the arsenoso compound.

The arsenoso compounds are crystalline materials, generally insoluble in water, sparingly soluble in al-

cohol, and more soluble in the less polar solvents like benzene. They are soluble in concentrated acids and alkali, but only slightly soluble in ammonia or alkali carbonates. Their melting points are usually more trustworthy than those of the arsonic acids, but the compounds decompose on strong heating into arsenic trioxide and tertiary arsines.



3. Arseno Compounds ($\text{RAs} = \text{AsR}$).

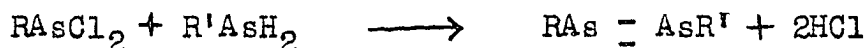
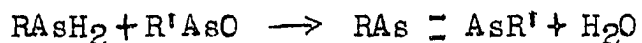
Exemplified by the important group of arsphenamines, these compounds are prepared by reduction of arsonic acids or arsenoso compounds, or by coupling reactions between primary arsines and arsenoso compounds or dichlorarsines to give pure or mixed arseno types. Reduction of arsonic acids can be done with sodium hyposulfite ($\text{Na}_2\text{S}_2\text{O}_4$) or hypophosphorous acid (H_3PO_2). The first reagent requires warming, and the resulting arseno derivative is somewhat contaminated with sulfur or sulfur-containing compounds. Nitro groups present in the original material are simultaneously reduced to amino groups. Thus, a well-known preparation of arsphenamine base involves the hyposulfite reduction of 3-nitro-4-hydroxyphenyl arsonic acid (46, 47).

Hypophosphorous acid, though more expensive, is a more rapid and cleaner reagent. Reduction of arsonic

acids may proceed in the cold or at room temperature, and nitro groups are largely undisturbed (in the absence of iodide). Other reducing agents such as stannous chloride in hydrochloric acid or sodium amalgam in alcohol have been used. Both of these latter reagents also reduce any nitro groups present.

The reduction of the arsenoso compound to the arseno form is effected by the same kind of reagents as are employed on arsonic acids. The reaction, however, proceeds more quickly, and low temperatures are feasible. Stepwise reduction of the arsonic acid to the arsenoso and finally to the arseno compound often results in products of superior quality and purity.

Condensations of aromatic primary amines with arsenoso or dichlorarsine groups can lead to unsymmetrical arseno molecules.

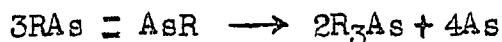


These unsymmetrical compounds can less satisfactorily be prepared by reduction of mixtures of arsonic acids or arsenoso compounds.

The arseno compounds are crystalline materials difficult to purify. They are, as a rule, insoluble in water, acids, alkalis, or common organic solvents. Solubility groups may be present to alter such properties

and are necessary for facile medicinal application. The arseno linkage is not as strongly chromophoric as is the analogous azo group and is, besides, more readily oxidized. Oxidation occurs slowly in the air, is more rapid in solution, especially in alkaline medium, and a variety of chemical oxidizing agents can readily convert them to arsenoso derivatives or arsonic acids. The presence of nuclear substituents generally increases the oxidative instability. The arsphenamines must usually be protected from oxidation by packaging in an inert atmosphere of nitrogen or carbon dioxide, and their solutions must be used within a short time after preparation. Toxicity of the mixture increases with the appearance of the arsenoso compound.

Melting points are generally unsatisfactory and the compounds decompose on strong heating to give tertiary arsines and elemental arsenic.



4. Quantitative Arsenic Determination.

Estimation of the arsenic content, following destruction of the organic matter, may be gravimetric or volumetric. The first usually involves the formation of magnesium ammonium arsenate which is ignited to the pyroarsenate and weighed as such. Intermediate purification may involve the formation of arsenious sulfide. Volumetric determination involves either the

titration, with standard thiosulfate, of the iodine liberated when an excess of potassium iodide is added to the acid solution of arsenic acid, or the iodine may be boiled away and the resulting arsenious acid titrated with standard iodine in a sodium bicarbonate buffer. Electrometric titration using standard bromate solutions has been employed with excellent results (22).

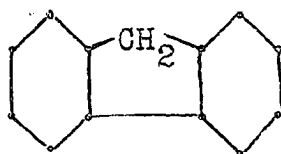
Oxidative decomposition of the sample is the preliminary operation in any event. This may involve fusion of the weighed sample in dry oxidation mixtures such as sodium peroxide, or potassium nitrate (70,72,77, 86,90,113). Such fusion-in-air procedures are said to give low results because of losses due to volatilization. Instead, an acid treatment may be used for decomposition. This may take the form of the usual Kjeldahl sulfuric acid-potassium sulfate-copper sulfate mixture (48,68,84) or the sulfuric acid may be combined with a more vigorous oxidizing agent such as fuming nitric acid, ammonium persulfate, or potassium permanganate (49,59,71,74,99). A recent review and evaluation of these methods was made by Banks, Sultzberger, Maurina, and Hamilton (4).

The method used by us is a modification of that of Robertson (98) and is as follows. A 0.2 gram sample is digested in concentrated sulfuric acid (6 ml.) and fuming nitric (2 ml.) at a temperature of 250°C. in a small Erlenmeyer flask (125 ml.) for $1\frac{1}{2}$ hours. An

additional 1 ml. portion of fuming nitric is added to the slightly cooled material and heating is continued for an additional half hour. The sample should be nearly colorless at this stage. An additional nitric acid treatment may be necessary. A one gram portion of ammonium sulfate is added to destroy the oxides of nitrogen, and the sample is cautiously diluted with water to about 70 ml.

One gram of potassium iodide is now added with one or two boiling stones. A clean, empty drying tube is clamped against the mouth of the Erlenmeyer flask with the small end of the tube down and the bulb fitting closely to the rim of the flask. The solution is evaporated to 40 ml. to drive off most of the iodine, and the light yellow color is destroyed by careful addition of 0.02 normal thiosulfate, and diluted quickly to 70 ml. with water. The solution is then poured into a 500 ml. Erlenmeyer flask containing 10 grams of sodium carbonate and 50 ml. of distilled water. An additional 5-7 grams of sodium bicarbonate and 2 ml. of starch solution are added and the whole is titrated with standard iodine solution. No blanks are necessary.

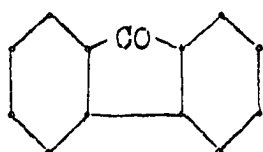
III. FLUORENE ARSENICALS.



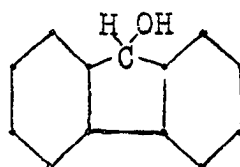
Fluorene

The hydrocarbon Fluorene, whose structural formula and accepted numbering system is given above, was isolated by Berthelot from crude anthracene oil in 1867 (13). It was named by him to suggest the beautiful fluorescence shown by the compound, especially in solution. The molecular formula, $C_{13}H_{10}$, was established by Barbier (6) and its structure finally elucidated by Fittig and Schmitz (53). It is, at present, available in 95% purity as a coal tar fraction.

Fluorene is a colorless solid of melting point 114 C. and boiling point 298°C (760mm.). It can be oxidized by dichromate and acetic acid to the keto compound fluorenone (65). Fluorenone, then, may be subsequently reduced to fluorenol with zinc dust and alkali (119) or sodium amalgam and alcohol (5,25). Thus, three series of closely related compounds are available for synthesis.



Fluorenone



Fluorenol

Nitration of fluorene with concentrated nitric acid in glacial acetic acid gives 2-nitrofluorene (14) which can be oxidized with sodium dichromate to the corresponding nitrofluorenone (30), or reduced with alcohol, zinc dust, and calcium chloride to 2-amino-fluorene (14). Nitration with fuming nitric acid gives a mixture of 2,7- (66%) and 2,5- (22%) dinitrofluorenes (2). These, similarly, can be converted by oxidation with chromic acid to fluorenones (30,82) or reduced to the diaminofluorenes with zinc dust and alcohol.

Nitrofluorenones may, in general, be reduced to aminofluorenones with ammonium sulfide (10,30) or amino-fluorenols with zinc dust and ammonia (26).

Direct chlorination with chloroform as solvent gives a mixture of 2-chloro and 2,7-dichlorofluorenes (63) while good yields of 2-chlorofluorene can be obtained in benzene solution using an iodine catalyst (18). Indirectly, 2-chlorofluorene may be obtained through Sandmeyer reaction on the 2-amino compound (28).

Similarly, bromination gives 2-bromo and 2,7-dibromofluorenes (24,115). And, indirectly, the 2-bromo compound is prepared through the diazo salt (27). Bromination in ultra-violet light gives largely the 9-bromo derivative (103).

The compounds 2-iodofluorene and 2-fluorofluorene

are made by diazonium reactions on the corresponding amines, the last involving the preparation of the fluoroborate (11,26).

Sulfonation gives first the 2-compound and then 2,7-fluorene disulfonic acid. Quantitative yields of the 2-sulfonic acid can be obtained in acetic anhydride as solvent (6). The 2,7-disulfonic acid is obtained by the action of concentrated sulfuric acid at 95 C. (108).

The Friedel-Craft reaction can be run normally on fluorene. Acetyl chloride with anhydrous aluminum chloride can be made to yield 2-acetyl or 2,7-diacetyl-fluorene (93) and similar reactions with benzoyl chloride or benzyl chloride yield corresponding compounds (55,57).

These are the main substitution reactions of fluorene. More extensive discussions of fluorene chemistry can be found in extant review articles (26,97).

It will be noticed that in all of the cases mentioned, substitutions of one ring with a group in the 2-position deactivates the ring so that the second entering group takes the 7-position exclusively except in the case of nitration when the 5-position is also, but to a lesser extent, attacked.

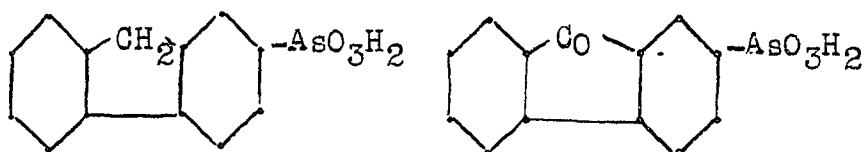
This tendency for a second entering group to locate itself on the unoccupied group is also strongly in evidence in the fluorenone series. For example, nitration

of fluorenone-4-carboxylic acid gives largely the 7-nitro compound with some small amount of the 5-nitro (78). The action of sulfuric acid at 250-260°C. on fluorenone gives 2,7-disulfonic acid (109). 2,7-dichlorofluorenone can be made from fluorenone with chlorine and a trace of iodine in chloroform (58), while the 2,7-dibromo compound can be made by the action of excess bromine on fluorenone (107). Similarly, 2,7-dinitrofluorenone results from the action of boiling nitric acid (d. 1.52) on fluorenone (108).

This fact of strong deactivation of one ring, especially in the case of a meta directing group, but even for ortho-para directing groups, will be of importance later in the discussion of certain structure proofs.

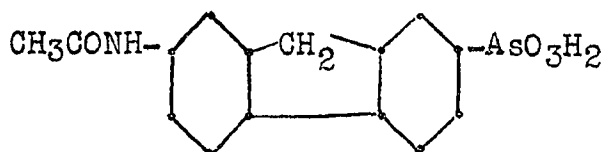
Now, no arsenic compounds of fluorene were reported in the literature previous to 1931. In that year, a paper by Cislak and Hamilton (23) described the preparation of three compounds of fluorene in which the arsonic acid group occupied the 2-position in the molecule. The first of these, fluorene-2-arsonic acid (I), was made through the Bart reaction of 2-aminofluorene with a yield of 19%. The second compound, fluorenone-2-arsonic acid (II), was made in 21.5% yield by essentially the same reaction on 2-aminofluorenone. The third compound

was made through a series of reactions starting with 2,7-dinitrofluorene. This was half-reduced with ammonium sulfide to 2-amino-7-nitrofluorene, the amino group was then protected by acetylation, and the compound was then reduced with zinc dust, alcohol and calcium chloride to 2-acetamino-7-aminofluorene. Reaction of the diazo salt of this with sodium arsenite in the usual Bart reaction gave a very low yield (2.5%) of 2-acetamino-fluorene-7-arsonic acid (III).



I

II



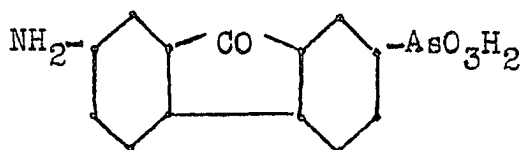
III

Analyses for arsenic were given, but no melting points. No mention was made of the possible medicinal use of these fluorene arsenicals, and no evaluation of their therapeutic properties was given.

In the same year, Morgan and Stewart (79) reported a similar synthesis of fluorene-2-arsonic acid and

fluorenone-2-arsonic acid. The compounds 2-fluorenyl-dichlorarsine, 2-arsenosofluorene, the corresponding compounds of fluorenone, and the semicarbazone of fluorenone-2-arsonic acid were briefly described. The sodium salts of the arsonic acids I and II were tested for therapeutic activity on trypanosoma equiperdum and found to have minimum lethal doses (M.L.D.) of 0.25 mgs. and 1.0 mg. respectively for 20 grams of mouse. No therapeutic (curative) action was found even in maximum doses.

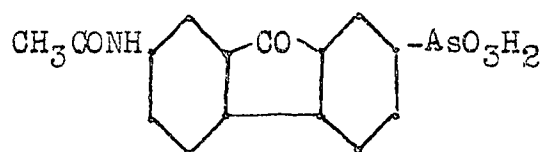
Also prepared was the compound 7-aminofluorenone-2-arsonic acid (IV) by nitration of fluorenone-2-arsonic acid followed by ferrous hydroxide reduction to the amine. No proof of structure was given here and reaction was merely assumed to follow analogous nitrations in the fluorenone series. The sodium salt of this arsonic acid showed a M.L.D. of 25 mgs. and a slight therapeutic action in maximum doses.



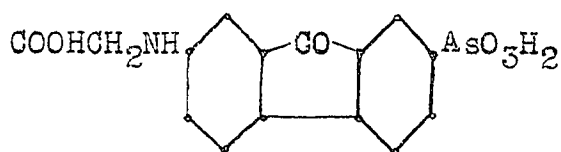
IV

Reaction of arsonic acid IV with acetic anhydride, chloracetic acid, and chloracetamide, gave compounds

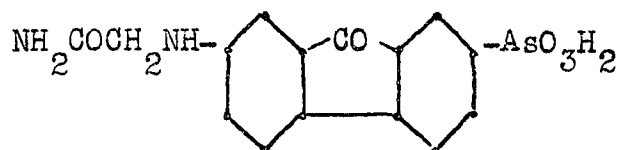
V, VI, and VII tabulated below with their therapeutic values.



V
M.L.D. 15mg.
M.C.D. 10mg.



VI
M.L.D. 50mg.
M.C.D. action only in
maximum doses.

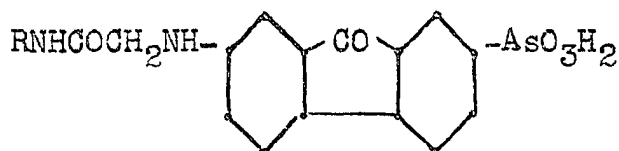


VII
M.L.D. 30mg.
M.C.C. action only in
maximum doses.

It will be noticed, as expected from similar observations in the benzene series, that the introduction of the amino group in the 7-position (corresponding to the para position in the case of benzene compounds) lowers the toxicity of the fluorene arsenicals. The compound 7-acetaminofluorenone-2-arsonic acid (IV) with a ratio of minimum lethal to minimum curative dose (therapeutic index) of 1.5 shows some promise.

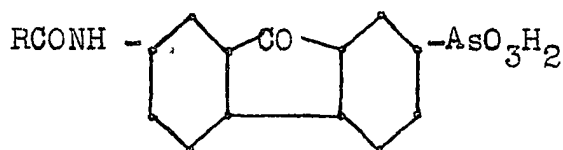
In a second paper (80), Morgan and Stewart described the preparation of several series of compounds

based on the 7-aminofluorenone-2-arsonic acid. Arsenicals of the type



were prepared in which R was successively methyl (CH_3-), phenyl (C_6H_5-) and p-acetophenonyl ($\text{CH}_3.\text{CO}.\text{C}_6\text{H}_4-$). All were prepared by the Jacobs and Heidelberger method (67,69) using the proper chloracetamide derivative in alkaline medium. The sodium salts of these glycine amide derivatives were again tested for trypanocidal action, the methyl compound showing a M.L.D. of 20 mg. and a slight curative power, the phenyl compound having little or no value, and the acetophenonyl derivative giving a M.L.D. of more than 50 mg. and no indication of curing.

A second series of compounds of the form

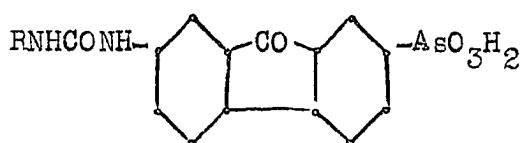


was investigated in which the compound R is CH_3- had already been prepared. By reaction of the corresponding acids, anhydrides, or acid chlorides with 7-amino-fluorenone-2-arsonic acid, four compounds were prepared

in which R was respectively H, ethyl (C_2H_5-), n-propyl ($\text{CH}_3\text{CH}_2\text{CH}_2-$), and phenyl (C_6H_5-). It was observed that curative powers are manifested when R is H, attains a maximum when R is CH_3- , and decreases rapidly when R is still further lengthened. No curative power is present when R is phenyl. The therapeutic results are tabulated (sodium salts on tr. equiperdum, mgs. per 20 grams of mouse).

<u>R</u>	<u>M.L.D.</u>	<u>M.C.D.</u>
H	20	20
CH_3-	15	10
CH_3CH_2-	5	5
$\text{CH}_3\text{CH}_2\text{CH}_2-$	1	nil
C_6H_5-	20	nil

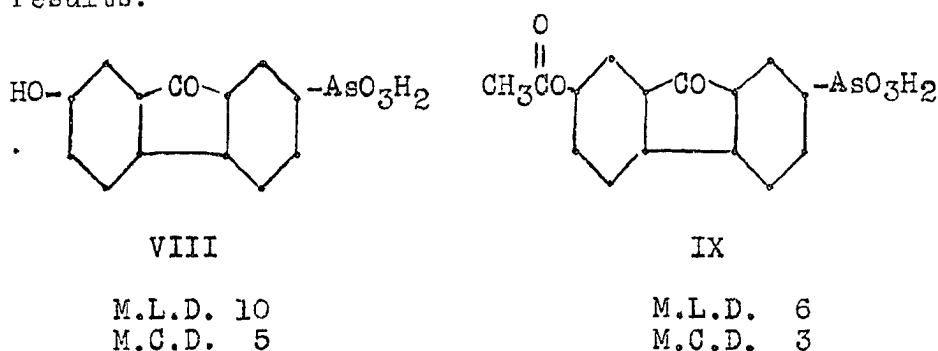
A third series of compounds of the substituted urea type was described.



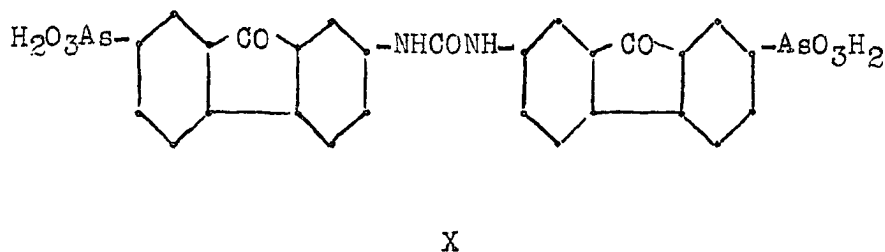
Arsenicals were prepared in which R is H, CH_3- , C_6H_5- (phenyl). The simple carbamido compound (R is H) in the form of its sodium salt gave a favorable therapeutic reaction with a M.L.D. of 30 and a M.C.D. of 15. The other two compounds were essentially non-curative. All

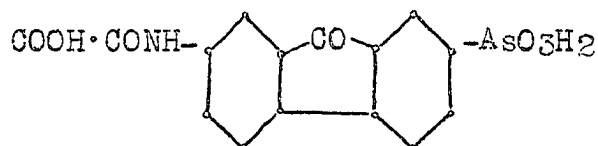
of these were prepared from 7-aminofluorenone-2-arsonic acid in alkaline medium through the action of potassium cyanate or the corresponding carbimides (CH_3NCO , $\text{C}_6\text{H}_5\text{NCO}$).

In this paper, also, was described the preparation of 7-hydroxyfluorenone-2-arsonic acid (VIII) by diazotization and hydrolysis of the 7-amino compound. Acetylation with acetic anhydride gave 7-acetoxyfluorenone-2-arsonic acid (IX). The sodium salts of these showed comparatively good curative action with the following results:

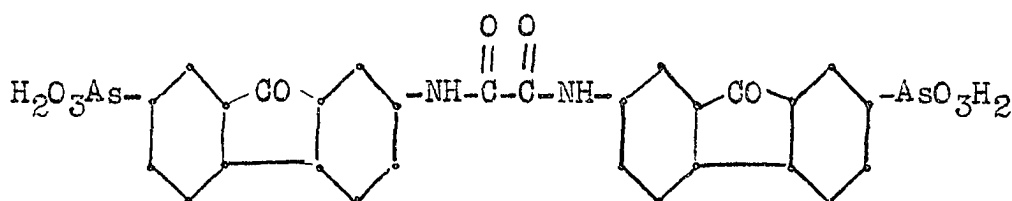


There were described, besides, the preparation of three disappointingly ineffective compounds, the first (X) prepared from 7-aminofluorenone-2-arsonic acid and carbonyl chloride, and the other two (XI and XII) by the action of oxalic acid.



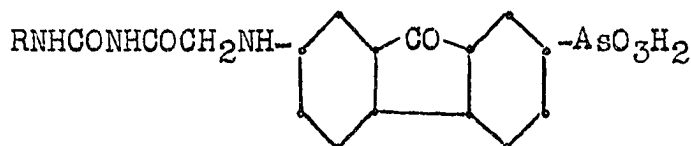


XI



XII

In a third paper (81), Morgan and Stewart investigated more fully the ureides corresponding to the following type.

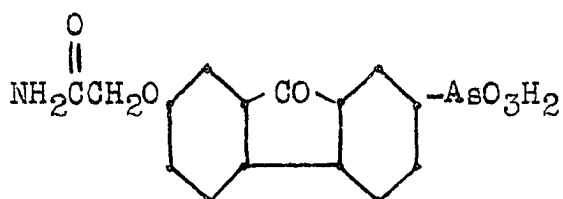


These were prepared from 7-aminofluorenone-2-arsonic acid, sodium iodide, sodium hydroxide and the corresponding chlorcarbimides ($\text{ClCH}_2 \cdot \text{CO} \cdot \text{NH} \cdot \text{CO} \cdot \text{NHR}$). An increase in the length of chain R rapidly destroys the therapeutic value of the compounds as can be seen from the following tabulation.

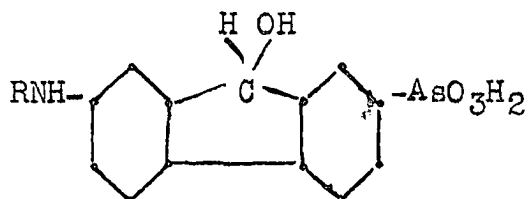
<u>R</u>	<u>M.L.D.</u>	<u>M.C.D.</u>
H	10	6
CH_3-	10	inactive
C_6H_5-	10	only slightly active

A better result was had from the action of chloracetamide on the 7-hydroxy arsonic acid to give 7-carbamylmethoxyfluorenone-2-arsonic acid (XIII) with a M.L.D. of 30 and a M.C.D. of 10.

In this last paper, the investigation of fluorenone arsenicals was also described (XIV). The 7-aminofluorenone-2-arsonic acid was obtained by catalytic reduction with hydrogen and platinum of 7-aminofluorenone-2-arsonic acid. By reactions already given, compounds were made in which R is $-\text{COCH}_3$, $-\text{CH}_2\text{CONH}_2$, $-\text{CONH}_2$, $-\text{CONHCH}_3$, and $-\text{CONHC}_6\text{H}_5$. These were evaluated as below:



XIII



XIV

<u>R</u>	<u>M.L.D.</u>	<u>M.C.D.</u>
H	9	curative in maximum doses
$-\text{COCH}_3$	30	15
$-\text{CH}_2\text{CONH}_2$	30-50	12.5
$-\text{CONH}_2$	15-30	some in max. doses
$-\text{CONHCH}_3$	12.5	slight
$-\text{CONHC}_6\text{H}_5$	12-20	slight

The above compounds complete the tabulation of fluorenone arsenicals already described in literature.

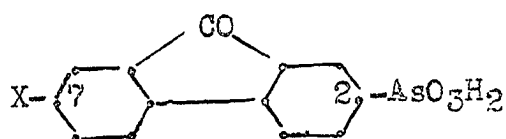
In summary, the most encouraging compounds so far uncovered are listed in Table V.

TABLE V

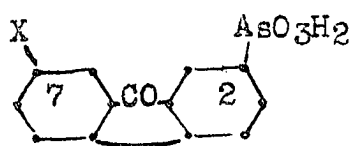
<u>Compound</u>	<u>M.L.D.</u>	<u>M.C.D.</u>	<u>Index</u>
7-acetaminofluorenone-2-arsonic acid	15	10	1.5
7-carbamidofluorenone-2-arsonic acid	30	15	2
7-hydroxyfluorenone-2-arsonic acid	10	5	2
7-acetoxyfluorenone-2-arsonic acid	6	3	2
7-carbamylmethoxyfluorenone-2-arsonic acid	30	10	3
7-acetaminofluorenol-2-arsonic acid	30	15	2
7-glycineamidofluorenol-2-arsonic acid	30-50	12.5	2.5-4

Obviously, then, the fluorene arsenicals thus far prepared offer little in the way of useful drugs. Therapeutic indexes should have a minimal value of 6 to 8 to be safely employed. Yet, at least, activity in some of them is fairly pronounced and, in view of the fact that only two relative positions, the 2 and 7, have been explored, further research is warranted. The 2,7 positions, viewed as substituents of the biphenyl system, correspond to the extended para, a form that would seem to offer the

most likely structure type in view of the corresponding



benzene analogs. Regarded as benzophenone derivatives, the relative positions become meta in character. Undoubt-



edly, the therapeutic values of various substituted biphenyl and benzophenone arsenicals might here suggest what types should be next attempted. But the literature is meager on the subject of arsenicals of these two parent compounds. It may be that the extended para position through the benzophenone portion of fluorenone, corresponding to the 3,6 series, will turn out to offer most. Easy entrance into these positions of the fluorene system is not yet possible.

A final consideration must be made. It has been shown (121) that certain of the fluorene derivatives possess carcinogenic qualities. It might be of interest to determine if fluorene-2-arsonic acid, like 2-acetylfluorene, possesses such activity. Very few arsenicals

have been evaluated carcinogenically as yet, and such evaluation would be limited to compounds fairly innocuous from the standpoint of arsenic content. In any event, even should these fluorene arsenicals show tendencies toward cancer formation, it is extremely doubtful whether such action would prohibit use of them as arsenicals. The course of arsenic treatment is short in comparison with that required for positive carcinogenic activity.

IV. NEW FLUORENE ARSENICALS

A. General Discussion.

The new arsenicals prepared by us are all of them fluorenone derivatives. Some extension of the 2,7 derivatives was first made, but the major portion of the work centered about the newly prepared parent arsenicals fluorenone-4-arsonic acid and fluorenone-1-arsonic acid. Finally, the Bechamp type reaction was attempted on three fluorenone amines: 2-aminofluorenone, 4-aminofluorenone, and 1-aminofluorenone. The availability of these amines was the greatest factor in determining choice of new derivatives. The preparation of these new compounds will be described in this order.

1. Derivatives of fluorenone-2-arsonic acid.

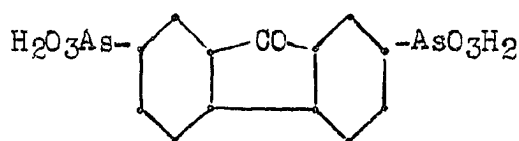
The compound 2-aminofluorenone was prepared by methods already described in the literature. Direct nitration of fluorene to give 2-nitrofluorene (14), followed by oxidation of the nitro compound to 2-nitrofluorenone (30), and reduction of this to 2-aminofluorenone was readily accomplished in good yield. We have made some improvement in the last reduction operation, using sodium sulfide-ammonium chloride-ammonium hydroxide in an alcohol medium and the details are given in the experimental section. For transformation of this compound to the corresponding arsonic

acid, the Scheller modification of the Bart reaction was successfully employed. Diazotization of the amine in acetone followed by decomposition with an excess of arsenic trichloride and a catalytic amount of cuprous chloride gave yields of product which consistently bordered on 40%. The Bart reaction, using an aqueous alkaline arsenite medium, generally gave yields some 20% lower. Use of acetone as the solvent medium for the Scheller modification appears to be an innovation, for no mention of its use in this regard was described in the literature. The more usual solvents, ethanol and glacial acetic acid, gave poorer results, especially in the case of acetic acid. Attempts to vary the conditions of the preparation or the mole-ratio of the reactants failed to improve the yield further.

Fluorenone-2-arsonic acid was then nitrated with fuming nitric acid-sulfuric acid to give the 7-nitro compound, and this was reduced to the corresponding amine with alkaline ferrous hydroxide according to the methods of Morgan and Stewart (79). Analysis for arsenic wherever possible gave close to the theoretical values.

A sample of 7-aminofluorenone-2-arsonic acid was transformed into the first of the new compounds by adding a water solution of the sodium salt containing sodium nitrite to a cold solution of sulfuric acid. The

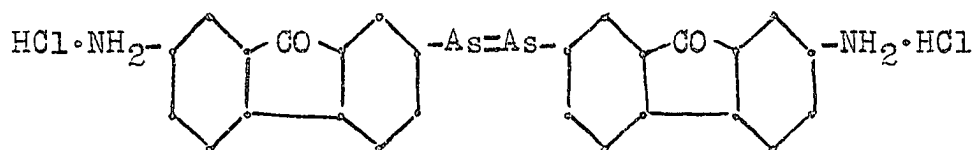
diazonium solution so obtained was then decomposed in regular Bart fashion by addition to an alkaline arsenite copper sulfate aqueous mixture. Purification of the insoluble material obtained by acidification gave a light yellow-brown material which analyses correctly, after purification, for fluorenone-2,7-diarsonic acid (I) in 67% yield. Purification was made through the sodium salt. The compound is somewhat soluble in hot water and glacial acetic acid. The strongly acid



I

character of the compound was indicated by the rapid effervescence when placed in dilute sodium bicarbonate solution at room temperature. The mono-arsonic acids of the fluorenone series react, in general, much more mildly under the same conditions.

A second portion of 7-aminofluorenone-2-arsonic acid was transformed into the corresponding arseno compound by warming a suspension of it in glacial acetic acid with an excess of hypophosphorous acid. The compound was converted into the double hydrochloride and analysed for arsenic as such (II). The product, a light brown



II

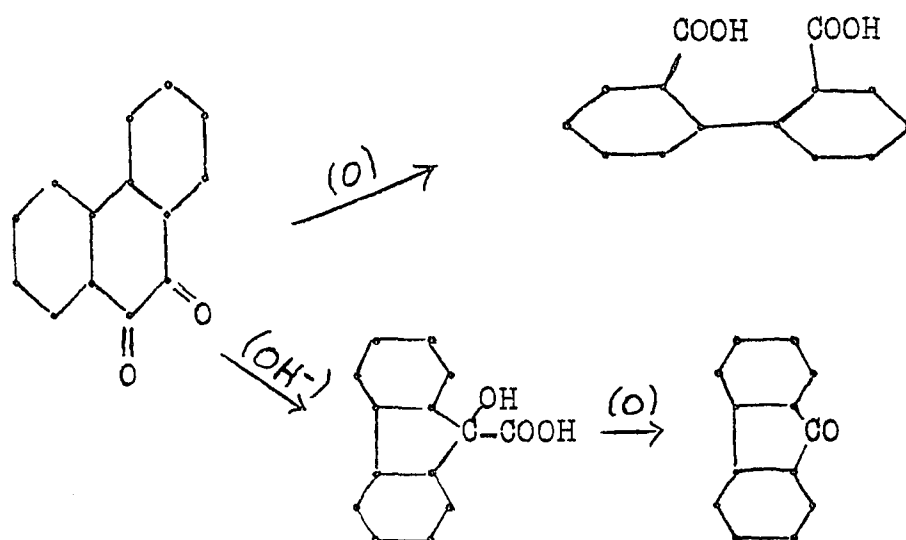
powder, was extremely sensitive to oxidation, turning dark on exposure to air, especially when wet with water or organic solvents. Its arsenic content diminished on standing, indicating transformation to oxygenated compounds. The solubility in water or in common organic solvents as the free base or hydrochloride is very limited. Treatment of the hydrochloride with dilute alkali liberated the yellow-brown di-amine. Diazotization of the amine followed by coupling with beta-naphthol in sodium acetate solution gave a bright red coloration intensified by the addition of some alcohol. No other arseno derivative in this series has been reported.

2. Derivatives of fluorenone-4-arsonic acid.

a. Preparation of 4-aminofluorenone.

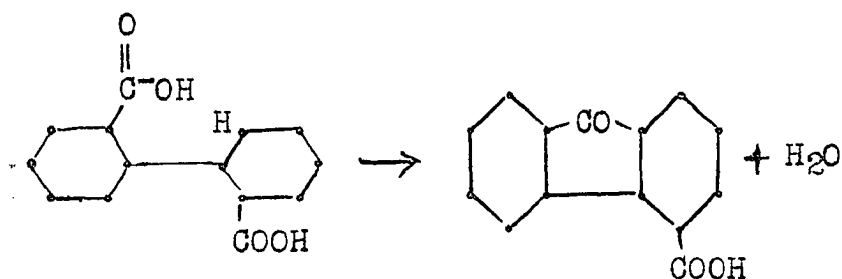
Recorded methods exist for the synthesis of 4-aminofluorenone starting with phenanthrene. Phenanthrene was oxidized in the usual manner (116) to phenanthraquinone using concentrated sulfuric acid and sodium or potassium

dichromate and purified through its soluble bisulfite addition compound. Oxidation of phenanthraquinone to diphenic acid (biphenyl-2,2'-dicarboxylic acid) can be done in a variety of ways. Use of sodium dichromate (116) or chromic anhydride (52) is common. A method employing alkaline permanganate (19) has been described. After much experimentation with conditions and varieties of alkaline media, the permanganate method has been used by us to give easy and reproducible conversion, the details for which will be found in the experimental section. Alkaline oxidation of phenanthraquinone may seem to be a less desirable procedure inasmuch as the benzilic acid rearrangement of the compound to biphenylene glycollic acid in dilute aqueous alkali allows of two concurrent reactions. Direct oxidation of phenanthraquinone yields diphenic acid, but oxidation of the rearranged compound results in fluorenone.



Having studied the effect of various temperatures and times of reaction, and a host of alkaline reagents (sodium carbonate, sodium bicarbonate, various sodium and potassium phosphates, sodium and potassium hydroxides, sodium acetate, etc.), it was finally discovered that barium hydroxide in suitable concentration afforded the proper medium for good conversion to the desired product. The advantage of the method is the formation of diphenic acid in good yield (65-70%), of high purity, in a short time (2-3 hours).

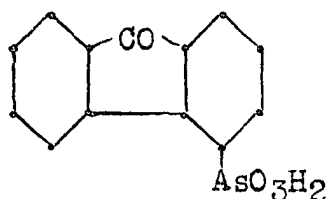
Diphenic acid is readily converted with concentrated sulfuric acid at 140°C . to fluorenone-4-carboxylic acid (66), and this can be transformed into the acid chloride



with phosphorus pentachloride (61), then into the amide by treatment with ammonia and finally into the corresponding amine by a Hofmann hypobromite reaction (66). Conversion of the acid to acid chloride can readily be accomplished also with thionyl chloride. The details for this modification are included in a later section. Transformation of the acid chloride to the amide was done in most cases with aqueous ammonium hydroxide.

b. Preparation of fluorenone-4-~~arsonic~~ acid.

This new parent arsenical (III) was prepared from the amine by a modified Scheller reaction carried out in acetone using a procedure pretty much the same as that used in the preparation of the 2-~~arsonic~~ acid. Yield of

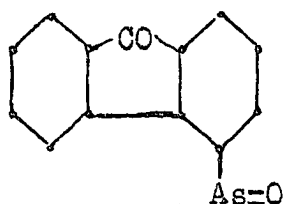


III

light yellow product was consistently 40%. The material was purified through the sodium or ammonium salt since the solubility of the free acid in hot water or common organic solvents is low. Arsenic determinations gave results that checked with the theoretical value. The crystalline orange-yellow monosodium salt was prepared by dissolving a portion of the free acid in hot water with the calculated quantity of sodium bicarbonate followed by the addition of several volumes of alcohol and subsequent cooling to effect crystallization. The disodium salt is readily soluble in water at room temperature to give an orange-red solution.

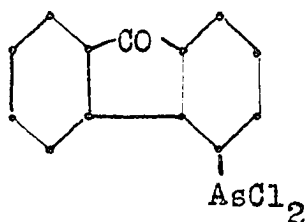
A portion of finely divided fluorenone-4-~~arsonic~~ acid was suspended in warm 6 normal hydrochloric acid, and a small quantity of potassium iodide was added.

The mixture was saturated with sulfur dioxide and allowed to stand over night. This treatment gave the light yellow amorphous arsenoso compound (IV) which was insoluble in water, alkali, and somewhat soluble in hot hydrochloric acid or hot glacial acetic acid. Analysis for arsenic confirmed the transformation. Another portion of



IV

fluorenone-4-arsenic acid was suspended in glacial acetic acid, refluxed to effect partial solution, and treated with a small quantity of phosphorous trichloride. Immediate dissolution was followed by a gentle reflux for a few minutes and, upon cooling, well-formed yellow crystals of the dichlorarsine (V) separated. These were purified by crystallization from glacial acetic acid, analysed for arsenic,



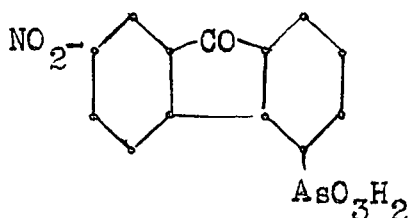
V

and submitted to melting point determination. The dichlor-

arsines of this series seem to be especially suitable for characterization. They are fairly stable crystalline materials, soluble in most inert solvents, and give good melting points. Most of the corresponding arsonic acids decompose on heating without melting.

c. Nitration of fluorenone-4-arsonic acid.

Samples of fluorenone-4-arsonic acid were nitrated in a mixture of sulfuric and fuming nitric acids at about 10°C. using a modification of the method of Morgan and Stewart (79). Yield of light yellow nitro compound was almost quantitative, and arsenic analysis checked with the value required for a mono-nitro fluorenone arsonic acid. Purification was made through the sodium or ammonium salts, these being readily soluble in water to give red-orange solutions. From a consideration of the chemistry of fluorene, the compound was taken to be 7-nitrofluorenone-4-arsonic acid (VI).



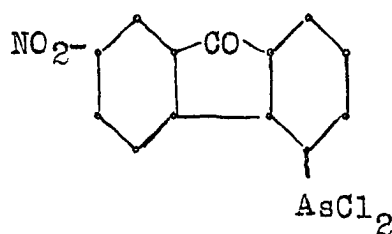
VI

d. Proof of structure of 7-nitrofluorenone-4-arsonic acid.

From a consideration of the orientation influences

present in fluorenone-4-arsonic acid, the likelihood for the formation of the 7-nitro compound by direct nitration is unquestionably high. All discoverable, analogous reactions of the series lead to this conclusion. It should be remarked that the compound obtained similarly by Morgan and Stewart from fluorenone-2-arsonic acid was tacitly assumed to be the 7-nitro derivative (79). Nevertheless, in order to establish structure beyond dispute, two methods of attack were followed.

In the first method, a small amount of the nitro-4-arsonic acid was converted to the dichlorarsine by a method similar to the one used in preparation of fluorenone-4-dichlorarsine. Again, beautiful yellow crystalline material resulted which was soluble in hot glacial acetic acid and analysed correctly for arsenic content. The melting point of this compound was found to be 231-232°C., and was the supposed 7-nitro-4-dichlorarsine (VII).



VII

A sample of this material was intimately mixed with mercuric acetate and submitted to fusion at temperatures

exceeding 300°C. according to the procedure of Skiles and Hamilton (112). Extraction of the melt with hot glacial acetic acid yielded a very small quantity of yellow crystalline material, supposedly the 2-nitrofluorenone. The melting point of this fusion product, together with melting points of pure 2-nitrofluorenone and a mixture of the two are tabulated.

	<u>M.P.</u>
fusion product	212-214°C.
2-nitrofluorenone	218-218.5°C.
mixture	215-217°C.

Such results are not conclusive, yet strongly suggest that the fusion product was indeed 2-nitrofluorenone contaminated with some starting material. The fusion product gave an evanescent Beilstein test for halogen. In view of the low yields from this procedure, and since the admittedly slim possibility of the nitro compound having the structure 2-nitro-4-arsonic acid would not be excluded, this method was discarded.

The second method was more successful. A sample of fluorenone-4-carboxylic acid was nitrated according to the method of Moore and Huntress (78) yielding mostly 7-nitrofluorenone-4-carboxylic acid with a small quantity of the 5-nitro compound. The 7-nitro compound was transformed by described procedures (78) through the chloride and amide into the amine. This 7-nitro-4-aminofluorenone was then converted by a modified Scheller procedure into

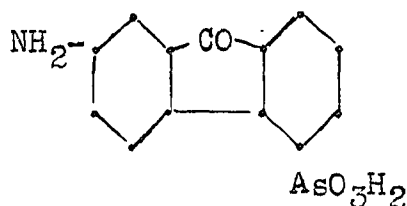
the corresponding arsonic acid in 43% yield. Conversion of this into the dichlorarsine gave 7-nitrofluorenone-4-dichlorarsine of unquestionable structure. The melting point of this compound, together with the melting point of the supposed identical dichlorarsine and a mixed melting point of the two are tabulated.

	<u>M.P.</u>
supposed 7-nitrofluorenone-4-dichlorarsine	231-232°C.
known 7-nitrofluorenone-4-dichlorarsine	231-232°C.
mixture	231-232°C.

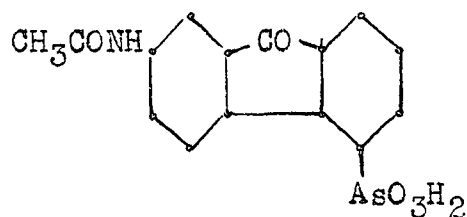
The product of direct nitration of fluorenone-4-arsonic acid has therefore shown to be 7-nitrofluorenone-4-arsonic acid.

e. Preparation of 7-aminofluorenone-4-arsonic acid and derivatives.

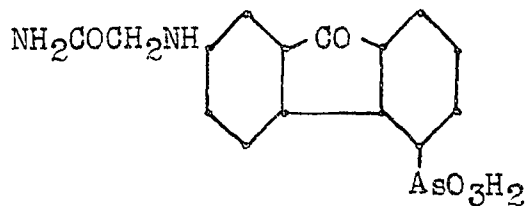
For the reduction of 7-nitrofluorenone-4-arsonic acid to the amine (VIII) alkaline ferrous hydroxide was again used. The isolated pale-brown material was readily soluble in dilute alkali, dilute hydrochloric acid, and coupled after diazotization with beta naphthol to give a red color. Arsenic analysis gave the proper values.



Two derivatives of 7-aminofluorenone-4-arsonic acid were prepared: the acetyl compound (IX) and the glycine-amide derivative (X).



IX



X

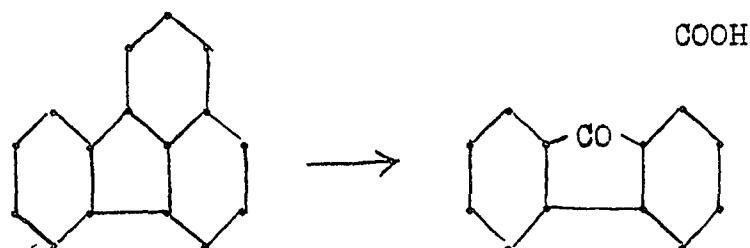
The first, a crystalline orange material, was readily made by stirring a suspension of the amine in excess of acetic anhydride at a temperature of 70-80°C. for one hour.

The second, a deep purple-red compound, was prepared by the addition of a slight excess of chloracetamide to a potassium salt solution of the amine arsonic acid, followed by a two to three hour period of heating at 90°C. A small amount of potassium iodide catalyzes the action. Correct arsenic analyses were obtained.

3. Derivatives of fluorenone-1-arsonic acid.

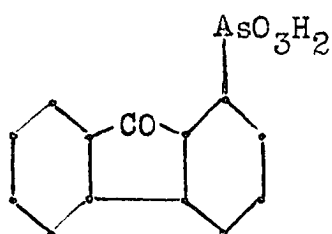
a. Preparation of fluorenone-1-arsonic acid.

Fluorenone-1-carboxylic acid can be obtained in a 1-step chromic oxide oxidation of fluoranthene (50). Purification



of the crude material was done through the sodium salt (12). The acid was then converted into the corresponding amine by the usual route through the acid chloride, amide, and Hofmann reaction (66). Again, thionyl chloride was used for conversion of the acid to acid chloride, and aqueous ammonia for conversion of acid chloride to amide. The final amino compound is of a gold-yellow color in sharp contrast to 2-aminofluorenone which is purple-red and the 4-aminofluorenone which is red-orange.

Conversion of 1-aminofluorenone into the corresponding arsonic acid (XI) was best accomplished (40-45% yield) by use



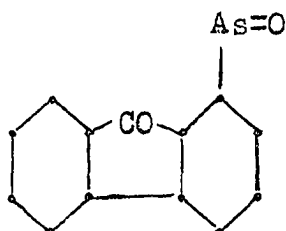
XI

of a modified Bart reaction. The amine was dissolved in warm acetone and the solution poured into an excess of dilute hydrochloric acid with efficient stirring. The partially precipitated amine and aminehydrochloride suspension was then chilled and diazotized in the usual manner. The diazonium solution was then added slowly to an alkaline arsenite-copper sulfate mixture and the reaction completed by warming. In this instance, the Scheller modification was found to give yields inferior by about 5-10%. The product was light yellow in color and, as usual, was purified through the sodium salt. Analysis for arsenic gave

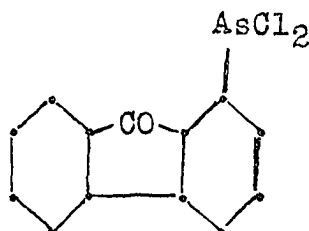
the expected values.

The monosodium salt of fluorenone-1-arsonic acid was made by the addition of some alcohol to a hot solution of the acid in aqueous bicarbonate, followed by cooling to effect crystal formation. A deep orange crystalline material was obtained, very soluble in water.

Using procedures similar to those employed in the 4-arsonic acid series, fluorenone-1-arsonic acid was converted into the corresponding light yellow, amorphous arsenoso compound (XII) and the deep yellow, crystalline dichloroarsine (XIII). This last, as was expected, was easily sol-



XII



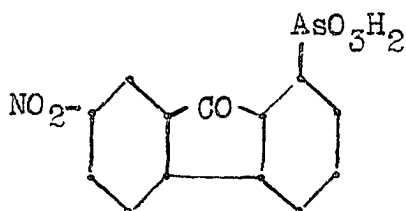
XIII

uble in glacial acetic acid and gave a fairly sharp melting point. Proper arsenic contents were found in both.

b. Nitration of fluorenone-1-arsonic acid.

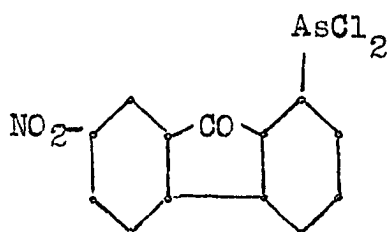
The procedure used for nitration of fluorenone-4-arsonic acid was employed for the nitration of the 1-arsonic acid. Again, yields of pale yellow material were practically quantitative, and correct arsenic analyses were obtained. Now, the problem of structure proof of this parent compound has not yet been solved. Again, from a consideration of

analogous cases, including the very similar one of nitrofluorenone-4-arsonic acid, it can be assumed that the compound is almost certainly 7-nitrofluorenone-1-arsonic acid (XIV).



XIV

In an effort to apply the mercuric acetate fusion technique to the problem, a portion of the nitro compound was transformed into the corresponding dichlorarsine using the procedure already mentioned; this new compound, the supposed 7-nitrofluorenone-1-dichlorarsine (XV), was then purified by solution in glacial acetic acid and analysed for arsenic. The melting point of the yellow crystalline



XV

material was 215°C. The results of the mercuric acetate fusion of the nitrodichlorarsine, however, were negative. No 2-nitrofluorenone could be isolated from the melt. Attempts to hydrolyze off the arsonic acid group with

60-70% by weight sulfuric acid were likewise without result. Work is progressing in several directions. The replacement of the arsonic acid group by hydrogen is generally a difficult operation. Apparently, the strength of bond is greater here than for the sulfonic acid group. Instances can be found in the literature where such a removal has been done with sodium amalgam in special cases (9), or by the action of hydriodic acid on the dichlorarsine. Such procedures are not, however, at all general and may fail in this instance. Reducing action would, of course, affect the nitro group as well, and in any event, the transformation into 2-nitrofluorenone would not absolutely eliminate 2-nitrofluorenone-1-arsonic acid from the possibilities, although it must be admitted that such a possibility is slim indeed. Along this line, a sealed tube method hydrolysis of the dichlorarsine with concentrated hydrochloric acid is being attempted.

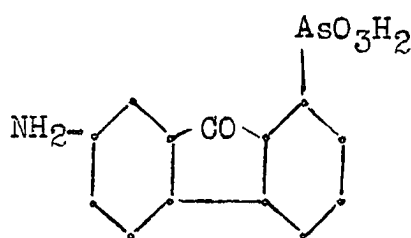
Replacement of the arsonic acid group by halogen can be made proceeding generally from the dichlorarsine to the organo-mercuric chloride, and then to the iodo or brome compound. But here, such a transformation is unfeasible in view of the lack of the corresponding known fluorene compounds.

Perhaps the most likely procedure would be to begin with the nitration of the 1-carboxylic acid of fluorenone, determine the structure of the nitrocarboxylic acid (probably the 7-nitro-1-carboxylic acid) and proceed then in a fashion similar

to the successful structure proof given above for 7-nitro-fluorenone-4-arsonic acid. Fluorenone-1-carboxylic acid has been nitrated (51) but structure determination of the product has yet to be made. Work along this line is in progress. For the present, the reasonable assumption will be made that the compound corresponds to formula XIV.

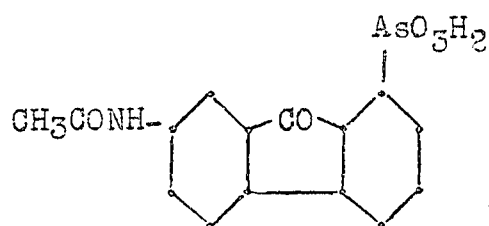
c. Preparation of 7-aminofluorenone-1-arsonic acid and derivatives.

The reduction of the nitro group of the supposed 7-nitro-fluorenone-1-arsonic acid was accomplished in the usual manner with alkaline ferrous hydroxide to give a pale brown material of proper arsenic content. This amino compound was readily soluble in alkali and dilute hydrochloric acid, and diazotized and coupled with beta naphthol to give a bright wine-red color. This compound is very probably, then, 7-aminofluorenone-1-arsonic acid (XVI).

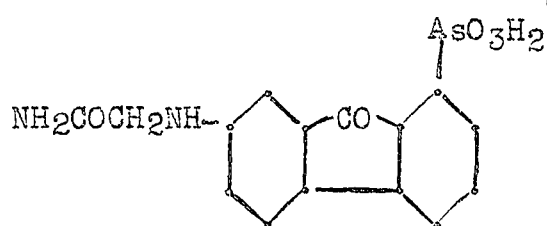


XVI

Using procedures similar to those employed in the 4-arsonic acid series, samples of 7-aminofluorenone-1-arsonic acid were transformed into a dark orange acetyl compound (XVII) and a dark purple-red glycineamide derivative (XVIII). These



XVII

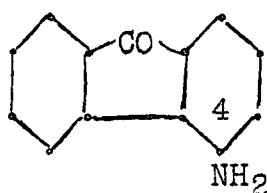
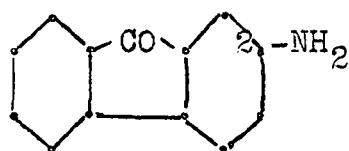


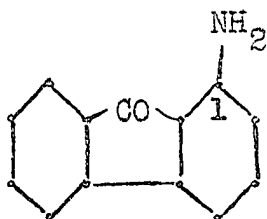
XVIII

were analysed in the usual manner. The free amine is less reactive towards acetic anhydride than is 7-aminofluorenone-4-arsonic acid and a higher reaction temperature for acetylation was used.

4. The Bechamp reaction on aminofluorenones.

The possibility of direct introduction of an arsonic acid group into the fluorene nucleus by treatment of a fluorene amine or phenol with arsenic acid according to the method of Bechamp was considered. The disadvantage of the procedure was seen to lie in the generally low yields obtained and in the necessity for subsequent structure proof. The advantage, on the other hand, is that an amine-arsonic acid is directly obtained and this could then be changed into a variety of amine derivatives in the usual fashion. No application of the Bechamp reaction to the fluorene series has yet been reported. The compounds selected for trial were 2-aminofluorenone, 4-aminofluorenone, and 1-aminofluorenone.





It will be observed that since the para position is blocked in the case of 2-aminofluorenone, only poor yields could be expected from the Bechamp reaction on this compound. Actually, after many attempts to carry out the reaction using a great many variations in method, no product could be isolated. Some of the original amine was recovered in almost every case, the remainder representing largely charred material from the oxidizing effect of hot arsenic acid.

Similarly, in the case of 4-aminofluorenone no amine-arsenic acid could be isolated from the reaction mixture. In this case, failure of the reaction was more surprising since the para position is open. On the other hand, the open para position is ortho to a meta-directing carbonyl group which may be effective in lowering the activity of that position.

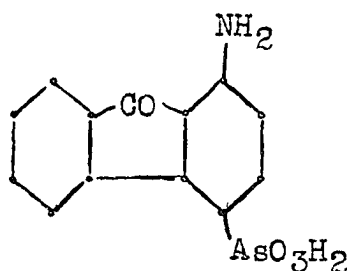
Now 1-aminofluorenone would seem to offer the best possibilities for a reaction of this type. Not only is the para position unoccupied, but that position is, besides, ortho to the benzene-benzene bond which activates the ortho

hydrogen to some degree. Moreover, the melting point of the amine is fairly low, $118^{\circ}\text{C}.$, making possible a fluid melt at the usual reaction temperatures (140 - $170^{\circ}\text{C}.$). In the first attempt, a product was isolated in low yields (about 5% based on the arsenic acid used) but of good appearance. The product was orange-yellow in color, readily soluble in alkali, slightly soluble in dilute hydrochloric acid, and tested for a free amino group by diazotization and coupling with beta naphthol. Subsequent improvements in procedure raised the yield to 8-10%. Solid arsenic acid, dried at $110^{\circ}\text{C}.$, was used with an excess of the amine and the mixture was heated at 160 - $170^{\circ}\text{C}.$ for three to four hours. The cooled melt was then extracted with dilute sodium hydroxide, and the amine arsonic acid was precipitated from the alkaline filtrate by careful acidification with dilute hydrochloric acid. Full details are to be found in the experimental section.

Structural proof for this compound has not yet been completed. The material does not give correct arsenic values, but even after purification through the sodium salt, yields results 2 to 5% low. The presence of some arsinic acid ($\text{R}_2\text{AsO}_2\text{H}$), a usual by-product of Bechamp procedures, would appear to explain the erratic analyses. Yet fractional precipitation of the compound from solutions of the sodium salt with dilute hydrochloric acid gives, first, a fraction that is lower in arsenic content than the value required for the

arsinic acid, while the more acidic fraction formed secondly gives results that approach the theoretical value for the amine-arsonic acid. The main portion is undoubtedly the expected product.

Both ultimate purification and proof of structure have yet to be made. For structural determination, deamination of the amino-arsonic acid through the diazonium salt to give the simple fluorenone arsonic acid; conversion of the arsonic acid to the dichlorarsine with its characteristic melting point; comparison of the melting point with that of the known fluorenone dichlorarsines, followed by a mixed melting point determination, would be fairly conclusive proof of structure. The possibility of attachment of the arsonic acid group to the unsubstituted ring can be ignored, and the compound is almost certainly 1-aminofluorenone-2-arsonic acid, or 1-aminofluorenone-4-arsonic acid. In view of the generally more facile substitution in the para position, the latter structure (XIX) is for the present assumed correct. But the 2-arsonic



XIX

acid cannot be rejected as a possibility in view of the fact that the Bechamp reaction on alpha naphthylamine gives the 2-compound (17).

B. Experimental.

Temperatures given in the following section refer to the Centigrade scale. Melting points were obtained with a calibrated thermometer using an aluminum block. Where melting points are not given in the case of the new arsenicals, it may be inferred that such melting temperatures were indefinite or above 300° .

1. Derivatives of fluorenone-2-arsonic acid.

2-aminofluorenone.

Fifty grams (0.222 moles) of 2-nitrofluorenone, 40 grams (0.753 moles) of ammonium chloride, one liter of alcohol, and 200 ml. (3 moles) of concentrated ammonium hydroxide were introduced into a 5 liter flask, fitted with reflux condenser and dropping funnel. The mixture was gently refluxed while a solution of 140 grams of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (0.583 moles) in about 400 ml. of water was slowly introduced. The mixture darkened at once and at the end of the addition (about 45 minutes) was an almost clear red. Refluxing was continued for one hour more.

The solution was now poured into 3 liters of water and let stand several hours (or overnight). The precipitate was filtered off and washed with water. It was then extracted by boiling with a solution of 150 ml. of concentrated (12 normal) hydrochloric acid in 1000 ml. of water. The low solubility of the hydrochloride required boiling tempera-

ture and filtration while hot. The insoluble material was extracted further with small portions of boiling dilute hydrochloric acid. The filtrates were combined and the residue, which was slight, was discarded.

The combined filtrates were neutralized with sodium hydroxide solution, and the precipitated amine was filtered off, washed with water and dried. The compound was sufficiently pure for most uses. A single crystallization from dilute alcohol gave a melting point of 162-163°. The average yield from a number of runs was 35 grams (80%).

Fluorenone-2-arsonic acid.

Ten grams (0.0512 moles) of finely divided 2-amino-fluorenone was dissolved in 400 ml. of acetone and 11.5 grams (0.112 moles) of concentrated sulfuric acid was added drop-wise with efficient stirring to precipitate some amine sulfate. The mixture was cooled to 5-10° and, with stirring, a solution of 5 grams (0.0725 moles) of sodium nitrite in 15 ml. of water was added over a period of ten minutes through a dropping funnel, the end of which was submerged in the acetone solution. The mixture, now more heavy with precipitated material, was stirred for thirty minutes more at a temperature not exceeding 10°, and 20 grams (0.110 moles) of arsenic trichloride was added all at once. Stirring and cooling was extended 30 minutes more when one gram of freshly prepared cuprous

bromide was added and the mixture was warmed slowly on the water bath to a temperature of 40° where evolution of nitrogen was brisk. After nitrogen evolution ceased (30 minutes) the material was poured, with stirring, into 1500 ml. of water and let stand for 2 hours. The insoluble portion was filtered off and extracted with a warm ($70-80^{\circ}$) solution of 15 grams of sodium bicarbonate in 800 ml. of water. A second extraction using 5 grams of sodium bicarbonate in 200 ml. of water was made and the filtrates were combined and treated with decolorizing carbon. The clear yellow filtrate was cautiously acidified with 6 normal hydrochloric acid until distinctly acid to litmus to give a light yellow precipitate of the arsonic acid. This was filtered off, washed well with water, and dried. The yield was 6.25 grams (40%).

For analysis, purification was made through the sodium salt using sodium bicarbonate solutions at $60-70^{\circ}$, acidifying with hydrochloric acid. The well-washed material was dried over a steam bath and then in an oven at $110-115^{\circ}$ for 6 to 7 hours or until constant weight had been obtained.

Arsenic calculated: 24.6% Arsenic found: 24.4%

7-nitrofluorenone-2-arsonic acid and 7-aminofluorenone-2-arsonic acid.

These compounds were prepared from fluorenone-2-arsonic acid using procedures of Morgan and Stewart (79).

Fluorenone-2,7-diarsonic acid.

Ten grams (0.0313 moles) of finely powdered 7-amino-fluorenone-2-arsonic acid was suspended in 100 ml. of water, and 5.6 grams (0.100 moles) of potassium hydroxide was added, and the mixture was warmed to effect solution. When cool, 2.3 grams (0.0334 moles) of sodium nitrite was added to the solution which was then cooled to 10° and introduced slowly into a mixture of 150 ml. of water and 15 grams (0.146 moles) of concentrated sulfuric acid held at 0°. The addition required about 45 minutes. Stirring was continued for 30 minutes more.

An alkaline arsenite solution was prepared by dissolving 35 grams (0.626 moles) of potassium hydroxide, 15 grams (0.0760 moles) of arsenious oxide and 1 gram of copper sulfate in 200 ml. of water. To this arsenite solution, the diazonium solution was added at room temperature in 10 ml. portions with stirring. Stirring was continued for 2 hours more, and the mixture was then slowly heated on a water bath to 90° until nitrogen evolution ceased. The material was filtered, the filtrate treated hot with decolorizing carbon, and the resulting clear yellow solution acidified with a slight excess of 6 normal hydrochloric acid to precipitate the fine yellow-brown diarsonic acid. This was filtered off, washed well with water, and dried to give 9 grams (67%) of material. For purification the product was washed with warm 1 normal hydrochloric acid and dissolved twice as the sodium

salt in dilute sodium bicarbonate solution with decolorizing treatment in each case and precipitation with dilute hydrochloric acid. For analysis, a sample of the material was dried in the oven at 110° for 7-8 hours.

Arsenic calculated: 34.7% Arsenic found: 34.2%

7,7'-diamino-2,2'-arsenofluorenone dihydrochloride.

A 1-gram portion of finely divided 7-aminofluorenone-2-arsonic acid was suspended in 10 ml. of glacial acetic acid, and 10 ml. of 30% hypophosphorous acid was added. The mixture was stirred in a water bath at $85-90^{\circ}$ for $2\frac{1}{2}$ hours. The buff-orange precipitate was filtered and transferred quickly to 100 ml. of boiled distilled water. The mixture was made alkaline with 2 normal potassium hydroxide, the brown free base was filtered and transferred to 100 ml. of 3 normal hydrochloric acid, and then filtered again as the buff-orange hydrochloride. The product was washed with dilute hydrochloric acid and dried in a vacuum desiccator over sodium hydroxide to give 0.65 grams (68%) of a light brown material which darkened upon heating in the oven for one hour at 110° . The compound was analysed as the dihydrochloride.

Arsenic calculated: 24.5% Arsenic found: 23.7%

2. Fluorenone-4-arsonic acid and derivatives.

Diphenic acid.

Sixty-five grams (0.412 moles) of potassium permanganate and 2 liters of water were introduced into a 5 liter, round-

bottomed flask, and the whole was heated until the permanganate dissolved. Then, 50 grams (0.240 moles) of finely powdered phenanthraquinone was added and the flask was swirled until the quinone was dispersed throughout the liquid. Technical $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (125 grams, 0.397 moles) was added and the flask again swirled until the hydroxide largely dissolved. The mixture was then refluxed for one hour, at the end of which time the quinone had mostly disappeared. A reflux period much beyond this was found to be disadvantageous.

The excess of permanganate was destroyed by the addition of a little alcohol, the mixture was filtered hot and the residue was extracted several times with small portions of hot water. The filtrate was then evaporated to about two-thirds the volume (about 1800 ml.) and finally treated with 2 grams of decolorizing charcoal. The material was then filtered, and the filtrate was acidified with dilute hydrochloric acid to precipitate a nearly white diphenic acid. The mixture was cooled to room temperature (or lower) and the acid filtered off, washed with cold water and dried. The average yield from several runs was 40.5 grams (70%) of melting point $229-230^\circ$.

Fluorenone-4-carboxylic acid.

The method of Graebe (61,66) was used on diphenic acid. It is suggested that the fluorenone-4-carboxylic obtained be washed several times by settling and decantation or siphoning before final suction filtration. The material is very

finely divided and slow to filter and wash on the funnel. Melting point of the material recrystallized from glacial acetic acid was 226-227°.

Fluorenone-4-carboxylic acid chloride.

Sixty grams (0.267 moles) of the finely powdered free acid was placed in a one liter round-bottomed flask fitted to a condenser arranged for downward distillation and a water trap to absorb liberated fumes. Then, 200 ml. (2.70 moles) of thionyl chloride was introduced in a slow stream through a dropping funnel. The mixture was warmed slowly in an oil bath until most of the gas evolution had occurred and a clear solution was obtained, when the excess thionyl chloride was distilled off and the temperature taken to 135° for 15 minutes. Application of suction during the final operation is advantageous but not essential.

After cooling, the solid product was loosened from the flask by gentle warming, the large lumps broken up in a mortar and the chloride recrystallized from benzene. The yield of yellow product was almost quantitative, melting point 127-128°.

Fluorenone-4-carboxylic acid amide.

A 50 gram portion of the finely divided acid chloride was added in portions with gentle stirring to 300 ml. of concentrated ammonium hydroxide, cooled in an ice bath, and the mixture let stand 4 hours or overnight. It was then filtered, washed well with water and dried. Yield of the

crude product was 41.5 grams (90%). Melting point after recrystallization from alcohol was 229-230°. For conversion to the amine, the crude amide was used.

4-aminofluorenone.

The hypobromite method of Huntress, Pfister and Pfister (66) was used on fluorenone-4-carboxylic acid amide. It was found that with runs on larger quantities of amide, the recorded yield of 74% could not be duplicated, but ran, instead, to 60-65%. The melting temperature of the 4-aminofluorenone after recrystallization from alcohol was 138-139°.

Fluorenone-4-arsonic acid.

The modified Scheller procedure used by us in the preparation of fluorenone-2-arsonic acid was employed. Yield of light yellow arsonic acid was 45%.

Arsenic calculated: 24.6% Arsenic found: 24.9%

The monosodium salt was prepared by dissolving a weighed quantity of the free arsonic acid (for example, 5 grams) in a minimum amount of warm water (about 50 ml.) with the calculated quantity (1.05 moles, 1.4 grams) of sodium bicarbonate. Dilution with an equal volume of alcohol or acetone resulted, after a time, in the formation of yellow-orange crystals of the salt containing an undetermined amount of water of hydration. The salt is readily soluble in cold water. After drying in the oven at 110° for 5 hours, arsenic analysis indicated the presence of about two molecules of water.

4-arsenosofluorenone.

A one-gram portion of finely divided fluorenone-4-arsonic acid was suspended in 25 ml. of 6 normal hydrochloric acid, and 0.1 gram of potassium iodide was added. On shaking, a slight iodine color was formed. The suspension was saturated at room temperature with sulfur dioxide, with occasional shaking, heated to boiling and the solution again saturated with the gas and allowed to stand overnight. The fluffy precipitate of the arsenoso compound was filtered off, washed with dilute ammonia and water, and dried. Yield of material was practically quantitative (about 1 gram). The arsenoso compound was washed several times with carbon disulfide, dried to constant weight at 110° and analysed.

Arsenic calculated: 27.7% Arsenic found: 27.6%

Fluorenone-4-dichlorarsine.

A one-gram portion (0.00328 moles) of finely divided fluorenone-4-arsonic acid was suspended in 10 ml. of glacial acetic acid and the temperature raised to the boiling point. Heating was interrupted and 1.5 ml. (0.0120 moles) of phosphorus trichloride dissolved in 5 ml. of glacial acetic acid was introduced with swirling. The arsonic acid quickly dissolved to yield a yellow solution. The material was then gently heated to near reflux for 5 minutes and allowed to cool to yield well-formed crystals of the dichlorarsine. The product was filtered, the yellow crystals washed with several small portions of cold glacial acetic acid and dried on a

steam bath. Yield was consistently high at 80% (0.85 grams). The melting point of the compound after recrystallization from glacial acetic acid was 161-163°. Further purification did not improve the melting point. The purified sample was dried at 110° for 2 hours and analysed.

Arsenic calculated: 23.1% Arsenic found: 23.2%

7-nitrofluorenone-4-arsonic acid.

A mixture of 32.5 ml. (0.585 moles) of concentrated sulfuric acid and 37.5 ml. (0.880 moles) of fuming nitric acid (d. = 1.50) was cooled in an ice-bath and 15 grams (0.0492 moles) of finely divided fluorenone-4-arsonic acid was introduced with stirring. During the addition, the temperature was not permitted to rise above 10°. The mixture was then stirred at room temperature for an additional hour and then poured into 500 ml. of ice water. The precipitated yellow product was filtered, washed with water and dried. Yield of crude material was 15.5 grams (90%). Purification was readily accomplished by dissolution in dilute ammonia, followed by charcoal treatment and precipitation with dilute hydrochloric acid. The pure nitro arsonic acid was dried in the oven at 110° for 7 hours for analysis.

Arsenic calculated: 21.6% Arsenic found: 21.5%

7-nitrofluorenone-4-dichlorarsine.

A 3 gram portion (0.00865 moles) of finely divided 7-

nitrofluorenone-4-arsonic acid was suspended in 30 ml. of boiling glacial acetic acid, and 2.5 ml. (0.0287 moles) of phosphorus trichloride dissolved in 5 ml. of glacial acetic acid was added slowly with swirling. The suspension cleared as the arsonic acid dissolved to give a yellow solution, and the mixture was then heated to near boiling for 5 minutes and allowed to cool. The fine yellow needles were filtered off, washed with ice-cold acetic acid, and dried. The product weighed 2.70 grams (85% yield). Purification was effected by recrystallization from glacial acetic acid to give a product melting at 231-232°. For analysis, the pure compound was dried at 110° for three hours.

Arsenic calculated: 20.3% Arsenic found: 20.5%

7-aminofluorenone-4-arsonic acid.

To 200 ml. of water containing 10 ml. of 6 normal sodium hydroxide was added 16.5 grams (0.0476 moles) of 7-nitrofluorenone-4-arsonic acid and the mixture was stirred and warmed to effect solution. A solution of 100 grams (0.488 moles) of commercial ferrous sulfate (containing about 3 molecules of water of hydration) in 400 ml. of water was slowly made alkaline by the introduction, with stirring, of 50% sodium hydroxide solution. To the suspension of ferrous hydroxide was added slowly, with stirring the solution of the nitroarsonic acid over a period of 30 minutes. The mixture was then warmed slowly on the water bath and maintained at 90° for one hour

with constant stirring. The material was then filtered by gravity and the brown-black precipitate of ferric hydroxide was washed thoroughly with 4 portions of boiling water. The combined deep red filtrates were acidified with 6 normal hydrochloric acid to the point of precipitation, and precipitation was completed by the addition of a little glacial acetic acid. The precipitated amine-arsonic acid was filtered off, washed with water, and dried to give 8.6 grams (56%) of light orange-brown product. For purification, the compound was dissolved in hot 3 normal hydrochloric acid, precipitated by careful neutralization with ammonium hydroxide and acetic acid, filtered, dissolved as the ammonium salt in warm, dilute ammonium hydroxide, precipitated as before with hydrochloric and acetic acids, filtered, washed with water and dried. For analysis, a sample was dried at 110° for 5 hours.

Arsenic calculated: 23.5% Arsenic found: 23.2%

7-acetaminofluorenone-4-arsonic acid.

To 8 ml. of acetic anhydride, 1.5 grams of 7-aminofluorenone-4-arsonic acid was added and the mixture was stirred in a water bath at $75-80^{\circ}$ for two hours. The amine gradually dissolved to give a dark red solution. The material was then poured into 100 ml. of ice water and let stand with occasional stirring for 15 minutes. The bright orange acetyl compound was filtered off and dried; the yield was practically

quantitative. The compound is somewhat soluble in hot glacial acetic acid. For analysis, a portion of the product was purified through the ammonium salt and dried at 110° for 5 hours.

Arsenic calculated: 20.7% Arsenic found: 20.2%

7-glycineamidofluorenone-4-arsonic acid.

A 2 gram portion (0.00532 moles) of 7-aminofluorenone-4-arsonic acid was mixed with 3.7 ml. of 2 normal potassium hydroxide solution and 5 ml. of water, then diluted with 15 ml. more of water and treated with 0.1 gram of potassium iodide and 1.5 grams (0.0161 moles) of chloracetamide. The clear wine-red solution was stirred in a boiling water bath for $2\frac{1}{2}$ hours to give a red-purple precipitate of the glycineamide derivative. The insoluble portion was filtered and washed with water. The dry yield was 2 grams (85%). The compound may be crystallized from alcohol by the addition of some ether. Fine purple crystals result. For analysis, the compound was purified through the ammonium salt and dried at 110° .

Arsenic calculated: 19.8% Arsenic found: 19.8%

3. Proof of structure of 7-nitrofluorenone-4-arsonic acid.

a. Mercuric acetate fusion of 7-nitrofluorenone-4-dichlorarsine.

The method of Skiles and Hamilton (112) was used. A 1.5 gram portion of the supposed 7-nitrofluorenone-4-dichlor-

arsine was intimately mixed with an equal amount of dry mercuric acetate and fused in a pyrex test tube set in a sand bath, the temperature being rapidly raised to 340-350° and maintained at that level for 7-8 minutes. After cooling, the test tube was crushed, and the charred material was finely ground and extracted with two 5 ml. portions of boiling glacial acetic acid. The extract was treated with charcoal, filtered, diluted with 2 ml. of water, and set to crystallize. Some 0.1 gram of yellow crystalline material was obtained which gave an evanescent Beilstein test for halogen. Melting point of the product was 212-214°. Melting point of pure 2-nitrofluorenone is 218°. A mixed melting point of the two materials gave 215-217°.

b. Proof of structure through 4-amino-7-nitrofluorenone.
4-amino-7-nitrofluorenone.

This compound was prepared from fluorenone-4-carboxylic acid using the procedures of Moore and Huntress (78). The pure orange-red compound, somewhat soluble in hot alcohol, glacial acetic acid, and acetone melts at 289-290°.

7-nitrofluorenone-4-arsonic acid.

A 4 gram portion (0.0167 moles) of finely divided 4-amino-7-nitrofluorenone was suspended in 200 ml. of acetone and the mixture was refluxed for 5 minutes, then immersed in an ice-bath and the temperature taken to 5° with mechanical stirring. Concentrated sulfuric acid (4 grams, 0.039 moles)

was added drop-wise, and a solution of 1.4 grams (0.019 moles) of sodium nitrite in 8 ml. of water was added over a period of 3-4 minutes. Stirring was continued for 30 minutes when 8 grams (0.0441 moles) of arsenic trichloride was added all at once and stirring extended an additional 30 minutes. A 0.2 gram portion of cuprous bromide was then added and the mixture was stirred at room temperature for $1\frac{1}{2}$ hours. The temperature was then raised to 40° on a water bath and maintained at that temperature for 1 hour when nitrogen evolution had ceased. The acetone was steam distilled away and 400 ml. of water was added to the residue. The solid material was filtered off, washed with water, and extracted with 500 ml. of warm water containing 4 grams of sodium bicarbonate. The filtrate was decolorized with carbon and acidified with 12 normal hydrochloric acid to give a yellow precipitate of the nitroarsonic acid. This was filtered off and dried to yield 2.5 grams of product (43%).

7-nitrofluorenone-4-dichlorarsine.

The method used previously for the preparation of dichlorarsines was here employed on 1.3 grams of the 7-nitrofluorenone-4-arsonic acid of known constitution. Yield was 1.1 grams (53%) which was purified as before by crystallization from glacial acetic acid, and dried at 110° for analysis.

Arsenic calculated: 20.3% Arsenic found: 20.6%

The purified nitrodichlorarsine melted at $231-232^{\circ}$ which corresponded to that of the nitrodichlorarsine prepared from

fluorenone-4-arsonic acid. A mixed melting point of the two gave 231-232°.

4. Fluorenone-1-arsonic acid and derivatives.

1-aminofluorenone.

Oxidation of fluoranthene to fluorenone-1-carboxylic acid was accomplished by the method of Fieser and Seligman (50) modified by the purification procedure of Bergmann and Orchin (12). The conversion of the carboxylic acid to 1-aminofluorenone was done using procedures of Huntress, Pfister and Pfister (66). Melting point of the pure amine was confirmed at 118-119°.

Fluorenone-1-arsonic acid.

A 15 gram portion (0.0768 moles) of 1-aminofluorenone dissolved in 150 ml. of acetone was poured into a mixture of 100 ml. of 12 normal hydrochloric acid and 400 ml. of water with efficient stirring. The amine was diazotized at 10° with a solution of 7.0 grams (0.096 moles) of sodium nitrite, added below the surface over a period of 20 minutes. Stirring was continued for 30 minutes more, and the addition of 1 gram of urea was made. After 15 minutes, the mixture was added in small portions to a solution of 15 grams (0.0760 moles) of arsenic trioxide, 15 grams of sodium bicarbonate, 50 grams of potassium hydroxide and 3 grams of copper sulfate dissolved in 600 ml. of water. The addition required 30 minutes and 6 normal sodium hydroxide was added as necessary

to maintain slight alkalinity. Benzene may be used to prevent foaming. The mixture was let stand 3 hours, slowly heated to boiling until nitrogen evolution was completed, filtered, and acidified with 6 normal hydrochloric acid. The pale yellow solid was filtered off, washed and dried to give 10.5 grams (45%) of the arsonic acid. Purification was accomplished through the sodium salt and the compound was dried at 110° for analysis.

Arsenic calculated: 24.6% Arsenic found: 24.9%

The mono-sodium salt was prepared as in the case of fluorenone-4-arsonic acid.

1-arsenosofluorenone.

The procedure used in the preparation of the 4-arsenos compound was repeated. Yield was 95% of light yellow compound.

Arsenic calculated: 27.7% Arsenic found: 27.9%

Fluorenone-1-dichlorarsine.

The method of preparation was substantially the same as that used for fluorenone-4-dichlorarsine. The yield of yellow crystalline material was 85%. Melting point was $138-140^{\circ}$.

Arsenic calculated: 23.1% Arsenic found: 23.3%

7-nitrofluorenone-1-arsonic acid.

Direct nitration of fluorenone-1-arsonic acid was effected as in the case of fluorenone-4-arsonic acid. Yield of yellow product was 90%.

Arsenic calculated: 21.6% Arsenic found: 21.1%

7-nitrofluorenone-1-dichlorarsine.

Prepared as in the case of the 4-derivative, the yield of yellow crystalline material was 80%. The product melted at 215-217°.

Arsenic calculated: 20.3% Arsenic found: 20.0%

7-aminofluorenone-1-arsonic acid.

7-nitrofluorenone-arsonic acid was reduced to the amine with ferrous hydroxide, using the procedure employed in the 4-arsonic acid series. A light brown compound was obtained in 55% yield.

Arsenic calculated: 23.5% Arsenic found: 23.1%

7-acetaminofluorenone-1-arsonic acid.

A 1.2 gram portion of 7-aminofluorenone-1-arsonic acid was treated at near reflux temperature for 2 hours with 10 ml. of acetic anhydride. The orange-brown solution was then poured into 25 grams of a mixture of ice and water and allowed to come to room temperature with occasional stirring. The yellow-orange product was filtered and dried. Yield of the acetamino compound was 0.6 grams (42%). Since purification through the ammonium or sodium salt seemed attended with hydrolysis, the compound was recrystallized from glacial acetic acid for analysis and dried at 110°.

Arsenic calculated: 20.7% Arsenic found: 20.5%

7-glycineamido-2-fluorenone-1-arsonic acid.

The compound was prepared as in the case of the corresponding 4-arsonic acid derivative. Yield of red-purple compound was 72%. For analysis, purification was effected through the ammonium salt.

Arsenic calculated: 19.9% Arsenic found: 19.7%

5. The Bechamp reaction on 1-aminofluorenone.Preparation of arsenic acid.

One hundred grams of pure arsenic pentoxide was refluxed with 500 ml. of water for one hour. The mixture was cooled and filtered from the small amount of insoluble residue. The clear solution was evaporated in a dish with frequent stirring until the concentrated liquor had transformed into a thick paste of crystals. The temperature in the evaporation process was kept above 100° , but was not permitted to rise above 115° . Upon cooling, the material solidified entirely, and it was ground and dried in the oven at 110° for 4 hours. It was then quickly transferred to a tightly sealed container.

Arsonation of 1-aminofluorenone.

A 6 gram portion (0.0308 moles) of dry 1-aminofluorenone was placed in a 60 ml. Pyrex test tube and the tube immersed some $1\frac{1}{2}$ inches in an oil-bath. The temperature was elevated to 145° and 3 grams of solid arsenic acid (0.0187 moles, calculated as $\text{H}_3\text{AsO}_4 \cdot \text{H}_2\text{O}$) was added over a period

of 10 minutes with continual hand-stirring. The temperature was then raised to 160-165° and maintained at that level for 2 hours and 15 minutes. At the end of the first 15-20 minute period, the mixture had thickened considerably and only occasional stirring was then applied. Moisture, collecting on the upper walls of the test tube, was removed by insertion of rolled filter paper from time to time. The material was removed from the oil-bath, cooled, and extracted with four 15 ml. portions of boiling 3 normal sodium hydroxide. The combined deep yellow filtrates were cooled and carefully acidified with 6 normal hydrochloric acid until slightly acid. The precipitated orange-yellow aminoarsonic acid, (probably 1-aminofluorenone-4-arsonic acid,) was filtered off, washed and dried to give 0.70 grams which corresponded to 11.7% yield based on weight of arsenic acid used. Purification as the sodium or ammonium salt yielded fractions which gave 3-5% low arsenic contents. A better purification was effected by the slow addition, with efficient stirring, of 1 normal hydrochloric acid to a solution of the sodium salt until about one-third of the material had precipitated. This was then filtered off, and precipitation was completed by the addition of more hydrochloric acid to the filtrate. The second fraction possesses an arsenic content that comes within 1-2% of the proper value.

V. SUMMARY AND CONCLUSION

Nineteen new fluorene arsenicals have been described. The chemical structures of fourteen of these have been established. For the remaining five compounds, the probable structures have been indicated.

The Bechamp reaction has been applied, for the first time, to the fluorenone amines, resulting, in the case of 1-aminofluorenone, in a new arsenical.

Improvements in the preparation of some of the intermediates have been reported, particularly in the case of diphenic acid and the use of the Scheller reaction for the preparation of fluorenone-2-arsonic acid. Acetone has been recommended as the solvent for Scheller reactions in preference to alcohol or glacial acetic acid.

The pharmacological values of the new compounds have yet to be ascertained. An attempt will be made to publish any significant fact discovered pertaining to the pharmaceutical value of the arsenicals. The sodium salts of two of them, fluorenone-1-arsonic acid and fluorenone-4-arsonic acid, have been submitted for screening.

It is hoped, in any event, that the chemistry of fluorene has been, to some degree, advanced.

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