to infinity the number of portions in which the oxidizing agent was added. This procedure is troublesome and of no advantage.

Expt. 10 shows that increasing the concentration of the organic matter in the mixture, while the concentration of alkali in the solution remains constant, decreases the yield obtained.

To determine whether or not *p*-nitrobenzoic acid was oxidized itself by alkaline permanganate, the following experiment was tried. Nine g. of *p*-nitrobenzoic acid, 985 cc. of water, 3 g. of sodium hydroxide, and 21 g. of permanganate (added in 5 separate portions) were boiled together under a reflux condenser. Each portion required about $^3/_4$ hour to become decolorized. When the solution had become colorless, the mixture was filtered and acidified, and there was recovered 6 g. of the acid. Three g. had, therefore, been burned up, which would theoretically reduce 24 g. of permanganate if converted entirely into carbon dioxide and water.

Summary.

A comparative study of experimental conditions, which have an influence on the oxidation of the isomeric nitrotoluenes by means of potassium permanganate in alkaline solution, have led to the following results:

(1) A gradually increasing concentration of alkali in the oxidizing mixture favors the oxidation of *o*- and *p*-nitrotoluene, up to a certain point, while the oxidation of the *m*-compound takes place best in an essentially neutral medium.

(2) An increasing dilution of the solution favors the oxidation of all the nitrotoluenes.

(3) *p*-Nitrotoluene is oxidized most readily, the *o*-compound next, and the *m*-derivative least.

In conclusion the writer desires to express his thanks for the constant and friendly interest of Professor Treat B. Johnson, under whose direction this work has been carried out.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH.]

AROMATIC ARSENIC COMPOUNDS. I.

A PLAN OF PROCEDURE FOR THE SYNTHESIS OF ARSENICALS FOR CHEMOTHERAPEUTIC RESEARCH.

BY WALTER A. JACOBS AND MICHAEL HEIDELBERGER.

Received July 2, 1919.

In the following communications and in those which will appear later, we shall describe certain groups of aromatic arsenic compounds which have been the subject of our studies for several years. In collaboration with Drs. Wade H. Brown and Louise Pearce, who have had charge of the biological phases of the work, we have prepared these substances for the treatment of experimental trypanosome and spirochaete infections.

Although the fundamental types of aromatic arsenic chemistry had been amply studied by Michaelis, the methods at his disposal were such as to limit considerably the development of the field opened up by his researches. The proof by Ehrlich and Bertheim[1] that "atoxyl," obtained by the arsenation of aniline,[2] is the sodium salt of *p*-aminophenylarsonic acid at once led to a great expansion in the study of aromatic arsenic compounds. Ehrlich and those associated with him developed the synthetic possibilities very fully and added greatly to our knowledge in this field. The substances described in their published accounts were mainly those containing one or more of the usual substituents such as amino, hydroxyl, carboxyl, halogen, nitro and the like on the same aromatic nucleus as either trivalent or pentavalent arsenic.

The great practical achievements of this work were the synthesis of diamino-dihydroxy-arsenobenzene (salvarsan base) and the discovery of its great value in the treatment of syphilis and other related diseases. Later synthetic attempts to improve upon this drug were mainly conducted along similar lines, involving the synthesis of substances analogous to salvarsan, of derivatives of the latter and of various metallic coördination compounds of these substances. In the majority of instances the studies have involved compounds in which the chemical variations have been confined to the aromatic nucleus containing the arsenic and it can be assumed that such material has been the subject of fairly exhaustive study.

It seemed wise, therefore, to look in other directions for opportunities for the further synthetic development of arsenic compounds rather than to attempt an extension of the already thoroughly studied groups of substances. However, in the formulation of any comprehensive and systematic developmental policy, it was necessary to keep in mind the biological aim of the work, and in planning our synthetic procedure we have accordingly given special consideration to certain desiderata which we believed essential in this connection. Since these requirements have influenced the character of the substances to be described in the following articles, we feel that a preliminary discussion of these considerations will be appropriate here.

In the first place, work in chemotherapy, to be logical, must be based on the assumption that there is a relationship between chemical constitution and biological action. The substances to be chosen should therefore be of such a character as to permit interpretation of the biological action as a function of chemical structure, in order that the data obtained may be used in the further prosecution of the work. This can be consistently carried out only when the compounds studied are sufficiently

[1] *Ber.*, 40, 3292 (1907).

[2] Béchamp, *Bull. soc. chim.*, 5, 518 (1863).

alike in general structure to reduce to a minimum the number of chemical features which must be considered in contrasting the biological properties of compounds in order to arrive at the chemical cause of the observed variations in action. The conditions for such homogeneity of material can best be realized by confining the studies to a group of closely related substances which may be regarded as derivatives of some parent compound. The various chemical modifications in the series of drugs would then be chiefly in the nature of the addition to the molecule of groups or side chains, readjustments in their relative positions or alterations confined to such groups or side chains.

In the selection of the types of substances due consideration should be given to the technical character of the work involved in their preparation. As the efficient prosecution of work in chemotherapy can be accomplished only by the intimate and parallel coöperation of both the biological and the chemical sides, preparative difficulties can not be allowed to consume a disproportionate amount of time and effort. It is desirable, therefore, that the types chosen represent readily accessible groups of substances. These types also should be of such a character as to permit of ready chemical modification by the addition of new groups or side chains which may in turn be altered at will as regards number, character, or position. It is important when a certain chemical modification is suggested as particularly desirable from a study of the biological results, that the modification be reasonably easy to execute. Otherwise, as has only too often occurred, the work will be brought to a standstill because of the difficulty of developing further a chemical lead which has been presented.

The realization of these requirements can be made easier if the substances chosen are prepared by reactions of a simple type. For this purpose as starting material there is chosen a readily obtainable substance which contains on the one hand the therapeutic element or radical, and on the other hand some reactive group such as, for example, the amino group. Each new compound would then be produced by the reaction of this starting material with a second class of substances, each of which possesses the same suitably reactive group as, for instance, aliphatically bound halogen. The production of the final compounds would in each case depend mainly on the accessibility of this second group of substances. It may not be irrelevant to point out that accessibility is not only an important aid to scientific orientation, but also determines the cost of any remedy which might be developed. Finally, for practical reasons, substances which can be dissolved in water are most desirable.

From a purely chemical standpoint, in applying these requirements to the preparation of arsenic compounds for biological study, it was obvious from the first that pentavalent arsenic compounds would offer the

best material for initial explorations. The trivalent arsenic derivatives as a class do not meet with the technical requirements as above set forth, whereas the more available, more easily handled, and more stable pentavalent compounds promised to afford wider opportunities for synthetic work. These substances, possessing the arsenic as the salt-forming arsonic acid residue, at once solved the problem of solubility. For biological work, arseno compounds, on the other hand, are restricted to those which possess other salt-forming groups and are therefore far more limited than the pentavalent compounds. We are inclined to regard reduction to the arseno form more as a chemical modification which could ultimately be attempted where the presence of a non-arsenical solubilizing group might make this desirable.

Arsanilic acid, the most accessible of the aromatic arsenic compounds, was naturally chosen as the fundamental substance. This compound contains not only the therapeutic element arsenic, in a salt-forming combination, but also carries a very reactive amino group. As Ehrlich and Bertheim have amply shown, it can be subjected to all the reactions which characterize primary aromatic amino compounds. It therefore afforded the logical and ideal starting material for the synthetic treatment outlined above. By choosing the proper reaction it was possible to make use of this amino group in such a way as to form a side chain which served the function of a connecting link between the benzene nucleus containing the arsenic and another group in which the chemical alterations were made. Moreover, in particular cases in which an optimal effect had been achieved with a substance derived from arsanilic acid, the opportunity always remained for studying the further effect of changes in position or of the addition of new groups in the nucleus containing the arsenic, by making the analogous substance derived from the isomers, homologs or other substitution products of arsanilic acid.

p-Hydroxy-phenylarsonic acid, another easily available aromatic arsenic compound, offered similar possibilities by virtue of the hydroxyl group, although this is more limited in reactivity than the amino group.

The following types which were developed in the present work will present this plan of procedure in more concrete form:

I. Diazoamino Compounds, $A-N=N-NRR'$, in which A is the arylarsonic acid radical and R and R′ hydrogen, alkyl, aryl or substituted aryl groups.

As Ehrlich and Bertheim have shown, arsanilic acid may be readily and quantitatively diazotized and the diazo compound coupled with appropriate couplers to form azo dyes. We have likewise found that the diazo compound couples in a normal manner with suitable secondary aliphatic amines and primary and secondary aromatic amines to form diazoamino compounds. The same result could also be obtained in the

case of the primary aromatic amines by first diazotizing these and coupling the resulting diazonium salts with arsanilic acid. The compounds obtained from the aromatic amines suited our purpose best.

II. Azo Dyes, A — N = N — R, in which A is the arylarsonic acid radical and R is the aromatic coupler.

III. *N*-Substituted Amides of *N*-Phenylglycine-*p*-arsonic Acid, A—$NHCH_2CONRR'$ in which R and R′ are hydrogen, alkyl, aryl or substituted aryl groups.

As a primary amino compound, arsanilic acid should react with alkyl halides to form *N*-alkyl derivatives. In the particular direction in which we chose to employ this reaction, its practicability had already been suggested by the preparation of the single substance, phenylglycine-*p*-arsonic acid, from arsanilic acid and chloroacetic acid.[1] We have demonstrated, as was to be expected, that this reaction could be extended to chloroacetyl compounds in general. Our attention was centered mainly upon the development of a series of compounds by the use of the chloroacetyl derivatives of primary and secondary aliphatic or aromatic amines.

IV. β-Substituted Ureides of *N*-Phenylglycine-*p*-arsonic Acid, A—$NHCH_2CONHCONHR$.—This type of substance is closely related to the previous one and was in like manner easily prepared from arsanilic acid and the chloroacetyl-uramino compounds, $ClCH_2CONHCONHR$ in which R may be hydrogen, or an alkyl, aryl, or substituted aryl group.

V. Substituted *N*-Phenylglycyl Derivatives of Arsanilic Acid, A—$NHCOCH_2NHR$.—Type III naturally suggests a parallel series of compounds in which the side-chain joining the two benzene nuclei is reversed. These substances were prepared with remarkable readiness from chloroacetylarsanilic acid and primary or secondary amino compounds.

VI. Substituted *o*-Phenyl Glycollyl Derivatives of Arsanilic Acid.—When the amine in the preceding series was replaced by a phenol in alkaline solution, chloroacetylarsanilic acid was found to react smoothly in the proper sense, yielding substituted *o*-phenyl glycollyl derivatives of arsanilic acid, of the following general formula: A—$NHCOCH_2OR$.

VII. Substituted Amides of *o*-Phenyl Glycollic Acid *p*-Arsonic Acid, A —*o*-CH_2CONHR.

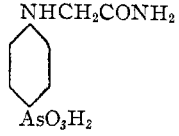
By a reaction identical with that given in Type VI, only starting with *p*-hydroxyphenyl arsonic acid and chloroacetylamino compounds, an analogous type of substances was prepared, differing from Type VI in that the side chain connecting the two nuclei is reversed.

Still other types were similarly studied.

Since the development of Type III has led to the most important results in the present work we shall confine the discussion to this group

[1] Ger. pat. 204,664.

of substances. In this type the fundamental member of the series is *N*-phenylglycinamide-*p*-arsonic acid,



This substance was prepared by the simple reaction of chloroacetamide on the sodium salt of arsanilic acid. By replacing the chloroacetamide by chloroacetylalkyl amines a limited series of compounds was prepared, which naturally preceded those of the aromatic series. The broadest scope for synthetic work, of course, was furnished by the aromatic series, compounds of this type being obtained from substituted chloroacetylarylamines and arsanilic acid.

This sub-group possesses two aromatic nuclei, an arsenical nucleus and a non-arsenical nucleus. It is apparent that by the choice of the proper chloroacetylarylamine any desired chemical groupings on the non-arsenical nucleus could be obtained. These could be made to vary at will as regards the character, number, and position of the substituting groups. And as the resulting substances were all obtained by the same reaction the technique of their preparation was easily standardized. The availability of any substance of this series was therefore determined only by the accessibility of the aromatic amine from which the chloroacetyl compound was prepared. As the alterations in the chemical groupings were first confined to the non-arsenical nucleus, each individual substance could be considered to be derived from the same parent type. Insofar as the biological action can be interpreted as a function of constitution, such a series therefore offered the opportunity of determining the biological value of each group, or of the position which it occupied in the nucleus. The correlation of the chemical and biological facts obtained with such homogeneous material offered a good opportunity for concluding in what direction new alterations within the same type should be attempted. And as the type was composed of available substances such dictates were generally possible to follow.

It was thus the purpose at first to confine the chemical alterations to the non-arsenical nucleus. But ultimately by employing a homolog, isomer, or other substitution product of arsanilic acid, modifications on the arsenical nucleus were attempted. Finally the side chain—$NHCH_2CONH$—which serves as the link between the two nuclei was also made the place for chemical alterations without destroying the relationship of the resulting compounds to the original type. This group could be changed to the chain—NHCHRCONH—in which R could be alkyl or aryl. Variations of this nature were generally made, not on a finished substance of Type III, but on an intermediate product; for

example, if R is phenyl, such compounds were prepared by reacting arsanilic acid with phenylchloroacetyl-amino compounds.

By the above treatment of the subject we have attempted to realize the conditions postulated at the start as essential in a logical plan for the synthesis of new arsenicals for biological study. It is our belief that a similar treatment of other leads, where chemically possible, will prove of service in chemotherapeutic investigations.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH.]

AROMATIC ARSENIC COMPOUNDS. II. THE AMIDES AND ALKYL AMIDES OF *N*-ARYLGLYCINE ARSONIC ACIDS.

BY WALTER A. JACOBS AND MICHAEL HEIDELBERGER.

Received July 2, 1919.

As in the case of other aromatic amino compounds, sodium *p*-aminophenylarsonate (sodium arsanilate) has been found to react with chloroacetic acid to form phenylglycine-*p*-arsonic acid.[1] In the present investigations we have found that the amide and alkyl amides of chloroacetic acid react in similar manner to form the amide and alkyl amides of phenylglycine-*p*-arsonic acid, with the following general formula

in which R and R′ may be hydrogen, alkyl, benzyl or substituted benzyl radicals. Although arsanilic acid itself may be employed in this reaction instead of the sodium salt, the reactivity of the amino group is suppressed by the negative arsonic acid radical so that the reaction proceeds very slowly, and satisfactory yields are only obtained when an extra molecule of the amino acid is employed, since the hydrochloric acid produced during the condensation renders a portion of the base inactive. On the other hand sodium arsanilate exhibits the full reactivity of the amino group and the sodium ion present neutralizes the hydrochloric acid as it is formed.

Chloroacetamide and the simpler chloroacetyl alkylamines condensed readily with sodium arsanilate in boiling aqueous solution, and although the reaction in no instance proceeded quantitatively, owing to the occurrence of side reactions, $^1/_2$ to 2 hours' boiling sufficed for obtaining optimum yields. In the case of the chloroacetylbenzyl amines, 50% alcohol was found to be the most serviceable medium owing to the spar-

[1] Ger. pat. 204,664.