

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF WISCONSIN.]

ACTION OF ARSENIUS CHLORIDE ON ANILINE.¹

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Introduction.

The action of arsenious chloride upon aniline has been studied by a number of investigators, each of whom has obtained different results. The reaction was first investigated by Schiff,² who described compounds resulting from the direct action of arsenious chloride and iodide on aniline, to which he assigned the formulas, $\text{As} \equiv (\text{NHC}_6\text{H}_5\text{HCl})_3$ and $\text{As} \equiv (\text{NHC}_6\text{H}_5\text{HI})_3$. Leeds³ obtained somewhat similar results, while Landau⁴ allowed arsenious chloride to react with aniline in benzene solution and obtained a compound to which he assigned the formula: $\text{AsCl}_3 + 4(\text{C}_6\text{H}_5\text{NH}_2) + \text{H}_2\text{O}$. Anschütz and Meyer⁵ were unable to obtain a compound corresponding to the one described by Schiff, and state that the third halogen of arsenic halide is not replaceable by aniline. They also show that the compounds described by Landau were probably nothing more than impure mixtures. They isolated two compounds, however, to which they assign the formulas, $\text{As}(\text{NHC}_6\text{H}_5)_2\text{Cl}_2$ and $\text{As}(\text{NHC}_6\text{H}_5)_2\text{Cl}$, and the corresponding arsenanilide dimethyl and diethyl esters.

More recently Morgan and Micklethwait⁶ describe a condensation product obtained by adding arsenious chloride to aniline in benzene solution. They assign to this compound either of the two formulas, $\text{C}_6\text{H}_5\text{NHC}_6\text{H}_4\text{-As}(\text{C}_6\text{H}_4\text{NH}_2)_2$, or $\text{C}_6\text{H}_5\text{NHC}_6\text{H}_4\text{As}(\text{NHC}_6\text{H}_5)\cdot\text{C}_6\text{H}_5\text{NH}_2$.

Substituted anilines have been more thoroughly investigated. Michaelis and Rabinerson⁷ found that dimethyl- and diethyl-aniline contain a reactive hydrogen atom readily replaceable by an arsenic radical, and in this manner prepared a number of substituted arsanilic acids.

In this research a study has been made of the action of arsenious chloride on aniline in *n*-heptane solution, with the result that a yellow granular substance was obtained, which could be purified by vacuum sublimation. This was apparently similar to Schiff's compound, except that it had a much higher melting point. The product showed interesting decomposition reactions, being transformed readily by heating it, either alone or preferably with an excess of aniline, into cyclic arsenic compounds.⁸ The

¹ Abstract of a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, University of Wisconsin.

² Schiff, *Compt. rend.*, **56**, 268, 1095 (1863).

³ Leeds, *THIS JOURNAL*, **3**, 134 (1882).

⁴ Landau, *Inaug. Diss.*, Berlin, 1888.

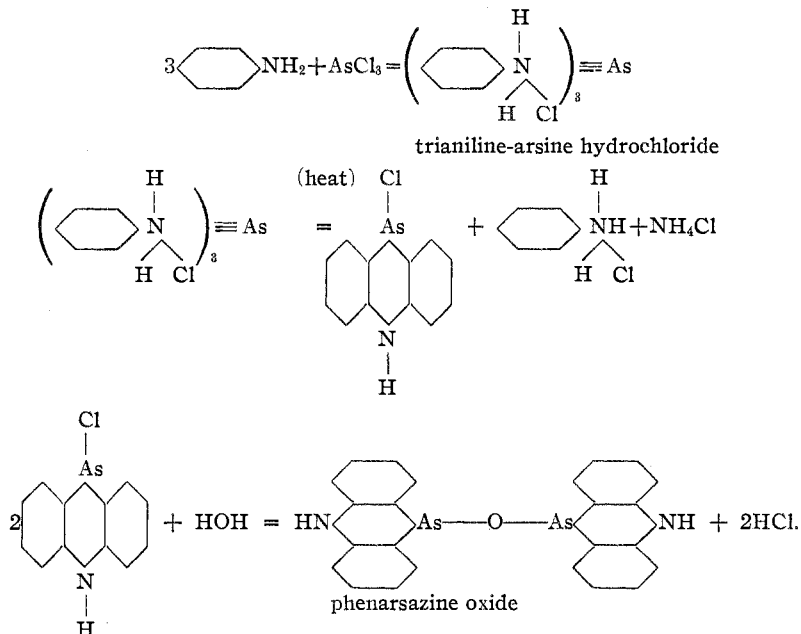
⁵ Anschütz and Meyer, *Ann.*, **261**, 279 (1890).

⁶ Morgan and Micklethwait, *J. Chem. Soc.*, **45**, 1473 (1909).

⁷ Michaelis and Rabinerson, *Ann.*, **270**, 139 (1892).

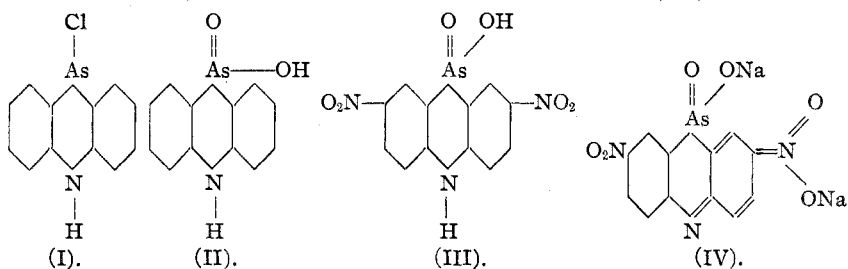
⁸ This work was completed previous to the publication by Wieland and Rheinheimer, *Ann.*, **423**, 1 (1921), on the preparation of similar condensation products obtained by the condensation of diphenylamine and arsenious chloride.

following equations probably indicate this transformation.

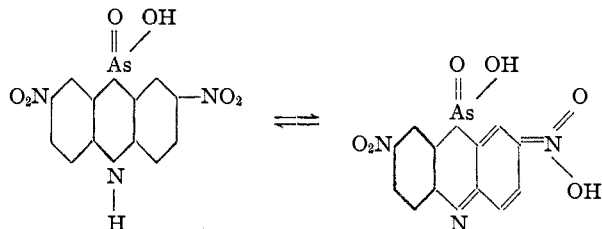


Chloro-phenarsazine, to which Formula I has been assigned, has been previously prepared⁹ by the action of arsenious chloride on diphenylamine.

Phenarsazine oxide was found to yield on oxidation the corresponding arsonic acid, (II) and on nitration a dinitro-arsonic acid (III). The latter



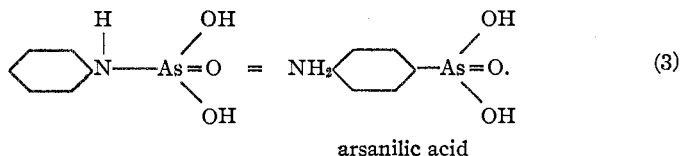
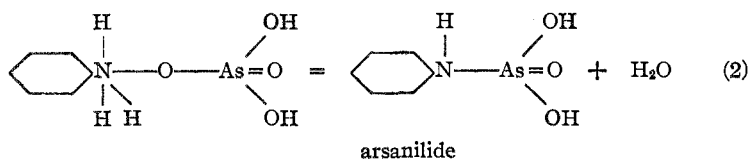
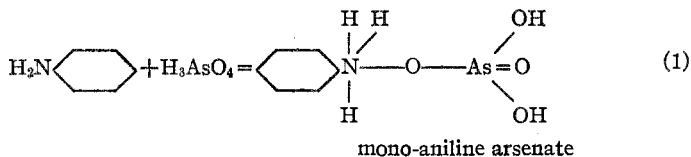
compound may evidently exist in two tautomeric modifications, which may be represented as follows.



⁹ Ger. pat. 281,049; *Centr.*, 86, 72 (1915).

This would satisfactorily explain the intense red color of alkaline solutions of the nitro compound. Further proof for this assumption has been obtained by the isolation of a di-sodium salt (IV) of the nitro derivative.

The study of the reaction of arsenious chloride and aniline was undertaken originally in an attempt to prepare "arsanilide," a substance which should be formed as an intermediate in the preparation of arsanilic acid by the reaction of aniline and arsenic acid according to the following equations.



The preparation of both a mono-aniline and di-aniline arsenate was easily accomplished, but it was found impossible to transform the former into an "arsanilide."

Experimental.

Trianiline-arsine Hydrochloride.—The heptane¹⁰ used was purified according to the method of Sherk¹¹ and dried over sodium. The aniline used was twice distilled and dried over sodium hydroxide. The arsenious chloride was a pure commercial material and not further purified.

Nine cc. of arsenious chloride (18 g. or $\frac{1}{3}$ mol) was added to 100 cc. of heptane contained in a large test-tube fitted with a mechanical stirrer and dropping funnel. The test-tube was kept well surrounded with ice. To the heptane solution of arsenious chloride 31 cc. of aniline ($\frac{1}{3}$ mol plus a slight excess) was slowly added with stirring. Immediate precipitation of a yellow granular compound occurred. After the complete addition of the aniline, stirring was continued for 15 minutes more and the reaction mixture then rapidly filtered with suction. The crude product was dried in a vacuum at 100°, since it holds heptane very tenaciously.

The ease with which the compound underwent decomposition and its insolubility in the common inert solvents (acetone, ether, benzene, and chloroform) made it extremely difficult to purify. Distillation in a vacuum was impractical, since the dis-

¹⁰ The heptane was kindly furnished by Dr. Kremers, Director of the Pharmacy Department, University of Wisconsin.

¹¹ Sherk, *Thesis*, University of Wisconsin, 1918.

tillate adheres so tenaciously to the receiver that it was often necessary to break the flask to obtain the product. Purification was finally effected by vacuum sublimation.

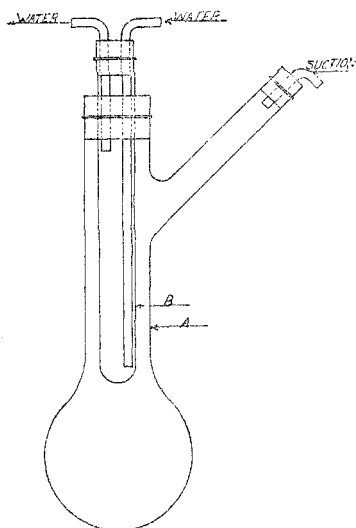


Fig. 1.

An apparatus was designed for this purpose, which is shown in Fig. 1. It consisted of a Beckmann boiling-point apparatus, A, in which a water-cooled test-tube, B, was inserted to serve as a condenser. The substance to be sublimed was placed in the outer flask, A, which was heated by a metal bath. The sublimate condensed on tube B to which it adhered firmly and was easily removed without loss.

The purified compound is a light yellow, crystalline solid melting with some decomposition at 148–150°, which is 60° higher than the melting point assigned to the compound isolated by Schiff. It is readily decomposed by water and absolute alcohol, dissolving in them to a clear solution when heated, but on cooling a mixture of arsenious oxide and aniline hydrochloride crystallizes out. The product undergoes oxidation on exposure to air and, unless immediately analyzed, gives somewhat low results for arsenic. Arsenic was determined by the method of Little, Cahen and Morgan,¹² chlorine by Stepanow's¹³ method, and nitrogen by the Kjeldahl-Gunning method.

determined by the method of Little, Cahen and Morgan,¹² chlorine by Stepanow's¹³ method, and nitrogen by the Kjeldahl-Gunning method.

Analyses. Calc. for $C_{18}H_{21}N_3Cl_3As$: N, 9.13; Cl, 23.10; As, 16.30. Found: N, 9.25, 9.21; Cl, 23.15, 23.00; As, 15.74, 15.75, 15.55.

Phenarsazine Oxide.—Five hundred cc. of dry redistilled aniline was placed in a 1-liter flask fitted with a dropping funnel and a reflux air condenser; 180 g. or 90 cc. of arsenious chloride was then added through the dropping funnel, a yellow precipitate of tri-aniline arsine hydrochloride being formed. On heating, the precipitate dissolved, the solution slowly darkening until finally it was deep purple in color. The temperature was slowly raised to the boiling point and boiling continued for 72 hours. While still hot the solution was poured into a 3-liter flask and 500 cc. of hot water carefully added. On cooling, the solution was made alkaline with sodium carbonate and the excess of aniline distilled with steam, the distillate being strongly alkaline, due to the ammonia formed in the reaction. The solution remaining in the flask was filtered away from the dark green powder which was washed several times with hot water, then with acetone and then again with hot water. This effectually removed the greater part of the impurities formed with the compound. For final purification it was recrystallized thrice from 80% acetic acid. When pure the compound was a pale yellow, crystalline solid, melting above 300°. The dry powder was extremely irritating to the membranes of the nose and mouth. The oxide was insoluble in alcohol, acetone, benzene, chloroform, ether, alkalis and dilute acids, but soluble in acetic acid and conc. sulfuric acid. The solution in sulfuric acid was deep purple in color, turning green upon addition of a trace of nitric acid. Water precipitated the original compound from its sulfuric acid solution. Yield of crude product, 70%. Analysis gave the following results, the arsenic and nitrogen being determined as in the previous experi-

¹² Little, Cahen and Morgan, *J. Chem. Soc.*, 109, 1356 (1916).

¹³ Stepanow, *Ber.*, 39, 4056 (1906).

ment, while combustion of the substance for determination of carbon and hydrogen was carried out in a tube filled with granulated lead chromate.

Analyses. Calc. for $C_{24}H_{18}ON_2As_2$: C, 57.60; H, 3.60; N, 5.60; As, 30.00. Found: C, 58.2, 58.09; H, 3.85, 3.57; N, 5.70, 5.74; As, 30.19, 30.05.

Phenazarsonic Acid.—Five g. of pure phenarsazine oxide was added to 100 cc. of water and 20 cc. of 15% sodium hydroxide solution. Ten to 20 cc. of perhydrol (30% hydrogen peroxide) was added and the mixture shaken and warmed until complete solution took place. The excess of hydrogen peroxide was decomposed by boiling, the solution filtered, cooled and acidified with acetic acid, the phenazarsonic acid being precipitated out. It was recrystallized from 50% acetic acid. It should be noted that pure phenarsazine oxide must be used, since certain impurities in the crude product completely prevent its oxidation by hydrogen peroxide. The pure product was a very pale yellow, crystalline solid, melting above 300°; yield 85%. It was readily soluble in dilute alkalis, insoluble in acetone, ether and alcohol.

Analyses. Calc. for $C_{12}H_{10}O_2NAs$: C, 52.36; H, 3.63; N, 5.09; As, 27.27. Found: C, 52.87, 52.37; H, 3.63, 3.74; N, 4.30, 4.83; As, 26.81, 26.93.

Dinitro-phenazarsonic Acid.—Five g. of pure phenarsazine oxide was heated under a reflux condenser with a mixture of 300 cc. of nitric acid (1:2) and 40 cc. of acetic acid until complete solution took place. The hot solution was filtered through glass wool into 2 liters of cold water, the nitro derivative being immediately precipitated. The crude product was purified by dissolving it in hot dil. ammonium hydroxide, filtering and reprecipitating the nitro compound with dil. hydrochloric acid; yield 80%. The pure product was a bright yellow powder which decomposed with a bright flash on heating. It was readily soluble in alkalis, forming a deep red solution, but sparingly soluble in water and alcohol. Analysis gave the following results, the nitrogen being determined by the modified Kjeldahl method for determining nitrogen in nitro compounds. Combustion of the substance was very difficult due to its explosive nature, the difficulty being finally overcome by distributing the substance in small portions along the boat and using extreme care in heating it.

Analyses. Calc. for $C_{12}H_8O_6N_2As$: C, 39.45; H, 2.19; N, 11.50; As, 20.54. Found: C, 39.92, 39.89, 39.40; H, 2.42, 2.44, 2.21; N, 11.8, 11.5; As, 20.16, 20.20.

The Sodium Salt of Dinitro-phenazarsonic Acid.—Two g. of the nitro compound was dissolved in the required amount of 0.025 *N* alkali to form the disodium salt. The solution was concentrated to a small volume on the water-bath, and on cooling the sodium salt crystallized out. It was recrystallized twice from alcohol, the pure product being a yellow crystalline solid, readily soluble in water, soluble in hot alcohol to form a bright red solution, but less soluble in the cold.

Analyses. Calc. for $C_{12}H_8O_6N_2AsNa_2$: As, 18.34. Found: 18.63, 18.35.

Mono-aniline Salt of Arsenic Acid.—This was prepared by dissolving redistilled aniline in alcohol, adding to it an excess of sirupy arsenic acid and warming until the aniline arsenate was in solution. On cooling, the mono-aniline salt crystallized in fine pearly leaflets. It was twice recrystallized from alcohol; m. p., 147–148°. Arsenic was determined by Ewins¹⁴ method and nitrogen by the Kjeldahl-Gunning method.

Analyses. Calc. for $C_6H_9O_4NAs$: N, 5.95; As, 31.91. Found: N, 6.22, 6.23; As, 31.67, 31.89.

Unsuccessful attempts were made to convert this salt into an "arsanilide" by heating it in either nitrobenzene, chlorobenzene or xylene. The use of dehydrating agents such as calcium oxide and copper sulfate also failed to give the desired result.

¹⁴ Ewins, *J. Chem. Soc.*, 109, 1356 (1916).

Di-aniline Salt of Arsenic Acid.—This was prepared by dissolving sirupy arsenic acid in alcohol, adding to it an excess of aniline and warming until the di-aniline salt was in solution. On cooling, it crystallized in pearly leaflets; m. p., 143°.

Analyses. Calc. for $C_{12}H_{17}O_4N_2As$: N, 8.53; As, 22.86. Found: N, 8.11, 8.10; As, 23.23, 23.32.

Summary.

1. The reaction between aniline and arsenious chloride has been studied and a new method for the preparation of secondary arsonic acid derivatives devised.

2. Tri-aniline-arsine hydrochloride has been prepared in a pure state and its properties described.

3. Several new compounds, phenarsazine oxide, phenazine-arsonic acid, dinitro-phenazine-arsonic acid, and disodium dinitro-phenazine-arsonic acid have been prepared and their methods of preparation and properties described.

4. A new vacuum sublimation apparatus has been described.

5. Directions for the preparation of mono-aniline and di-aniline salts of arsenic acid have been given.

The writer wishes to express his gratitude to Professor Richard Fischer, under whose direction this work has been carried out, and also to Professors Loevenhart and Kremers, for their interest in this research.

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EFFECT OF CERTAIN ANTISEPTICS UPON THE ACTIVITY OF AMYLASES.

BY H. C. SHERMAN AND MARGUERITE WAYMAN.

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In experimental studies of enzyme action, antiseptics are frequently used to prevent or suppress any activities of microorganisms. It is obviously important to know as definitely as possible whether antiseptics can be thus used without influence upon the activity of the enzyme which it is desired to study; and furthermore we may hope that a systematic determination of the behavior of enzymes toward antiseptics of different types may ultimately throw light upon the chemical nature of the enzymes themselves and the discussion of enzyme dispersions in terms of colloid chemistry.

The experiments here recorded have to do with the influence of toluene, formaldehyde, and copper sulfate upon amylases of both animal and vegetable origin and when tested in both natural and purified form. The literature affords many statements of a general nature regarding the relation of antiseptics to enzyme action, usually giving the impression that