CHEMOTHERAPY OF TRYPANOSOME AND SPIROCHETE INFECTIONS.

CHEMICAL SERIES. I.

N-PHENYLGLYCINEAMIDE-*p*-ARSONIC ACID.

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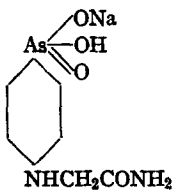
For a number of years the writers, in conjunction with Dr. Wade H. Brown and Dr. Louise Pearce, have been engaged in the synthesis of certain new types of organic arsenic compounds for the treatment of experimental trypanosome and spirochete infections. Although considerable of our effort has been given to the study of trivalent arsenicals our attention was at first confined to the development of compounds containing arsenic in the pentavalent form as presented by the arsonic acids. This group seemed best suited for the synthetic procedure which was employed and afforded the best opportunity for obtaining such information as to the relationship between chemical structure and biological action as would be useful for further work. Again it was felt that if a practicable and efficient drug could be found within this group it would go far toward eliminating certain of the well known practical disadvantages of the usual arseno compounds, for as an arsonic acid it should form soluble and stable salts and perhaps offer fewer of the preparative uncertainties than have been the experience with trivalent arsenicals.

In the course of these studies a number of substances have been prepared which have given interesting experimental results. However, those obtained with one in particular, the sodium salt of *N*-phenylglycineamide-*p*-arsonic acid



were such as to demand special attention. The simplicity of this compound, the ease of preparing it, its relatively inexpensive character, stability, and solubility, and its favorable biological behavior, warrant a publication of results. We wish therefore to present in conjunction with the following biological papers of Dr. Brown and Dr. Pearce, the facts as to the preparation and properties of the substance which will be appropriate in the present place. A somewhat fuller description of the substance and of related compounds has appeared elsewhere.[1]

Phenylglycine-*p*-arsonic acid, and its homologue, *o*-methylphenylglycine-*p*-arsonic acid, have already been described in German Patent, No. 204,664. These substances, of interest solely as the source of the arsenophenylglycines which were obtained by their reduction, were readily prepared by the interaction of the sodium salts of the amino-arylarsonic acids and sodium chloroacetate. In our investigations we have found that a reaction of this type, with α-halogenacylamino compounds, XCH_2CONHR, instead of the α-halogen acids, XCH_2COOH, is capable of practically unlimited extension and has rendered possible the preparation of a new series of aromatic arsenic compounds. In these substances the free carboxyl group of the above glycines has been changed to the amide or substituted amide group.

N-Phenylglycineamide-*p*-arsonic acid,[2] prepared by the methods to be described in the experimental part, readily yields a colorless,

[1] Jacobs, W. A., and Heidelberger, M., *J. Am. Chem. Soc.*, 1919, xli, 1587.

[2] This substance and related compounds to be described elsewhere are covered by United States Patents, Nos. 1,280,119–1,280,127. Patents have also been applied for in foreign countries.

crystalline sodium salt which is extremely easily soluble in water, forming neutral solutions, which are perfectly stable. In fact a 10 per cent solution may be boiled a reasonable length of time without appreciable cleavage of ammonia or arsenic.

The materials required and the method of preparation are such that the large scale production of the substance should offer little difficulty.

EXPERIMENTAL.

N-(Phenyl-4-Arsonic Acid)-Glycineamide (N-Phenylglycineamide-p-Arsonic Acid).—Of the two methods used for the preparation of this substance that described first is more direct.

434 gm. of arsanilic acid were dissolved in 2 liters of normal sodium hydroxide solution. After the addition of 375 gm. of chloroacetamide the mixture was boiled under a reflux condenser for 45 minutes, the clear solution setting to a solid mass of the crude product on cooling. 75 cc. of concentrated hydrochloric acid were added to the cold mixture to hold any unchanged arsanilic acid in solution and the substance was then filtered off and carefully washed with cold water. For purification it was suspended in sufficient water to form a thin paste and carefully treated, with stirring, with 25 per cent sodium hydroxide solution until the acid was completely dissolved. The filtered solution was then treated with an excess of acetic acid whereupon the substance separated as minute, lustrous plates. After filtering, washing thoroughly, and drying, the yield was 300 gm.

The acid is very sparingly soluble in cold water but dissolves readily on heating. It separates from the hot aqueous solution in aggregates of long, thin plates. It is insoluble in methyl alcohol, acetone, or chloroform and sparingly in hot methyl or ethyl alcohol, but dissolves in boiling acetic acid. It is sparingly soluble in dilute

hydrochloric acid but dissolves readily in the strong acid, its behavior showing it to be a weaker base than arsanilic acid. On boiling its solution in sodium hydroxide ammonia is evolved. When rapidly heated in an open capillary tube it darkens and softens at 280°C., but does not melt.

0.1405 gm. of substance; 12.35 cc. of N (22.0°C., 761 mm.).
0.3205 gm. of substance; 0.1832 gm. of $Mg_2As_2O_7$.
Calculated for $C_8H_{11}O_4N_2As$: N, 10.22 per cent; As, 27.33 per cent.
Found: N, 10.18 per cent; As, 27.59 per cent.

Sodium Salt.—The pure acid is suspended in enough water to form a thick paste and carefully treated with 25 per cent sodium hydroxide solution until completely dissolved and the solution reacts neutral to litmus. Two volumes of alcohol are then added, the pure sodium salt quickly separating as thin, nacreous plates. After filtering and washing with 85 per cent alcohol it is air-dried and then contains one-half molecule of water of crystallization. The sodium salt is extremely soluble in cold water, the solution reacting neutral to litmus. The apparatus, filter paper, water, etc., used in the preparation of this salt must be free from calcium; otherwise a precipitate of the insoluble calcium salt will contaminate solutions of the sodium salt.

0.3921 gm. of air-dry substance, at 100°C. *in vacuo* over H_2SO_4; 0.0117 gm. loss.
Calculated for $C_8H_{10}O_4N_2AsNa.\ \frac{1}{2}\ H_2O$: H_2O, 2.95 per cent.
Found: H_2O, 2.98 per cent.
Anhydrous: 0.1503 gm. of substance; 12.45 cc. of N(25.0°C., 762 mm.).
0.2300 gm. of substance; 0.1195 gm. of $Mg_2As_2O_7$.
Calculated for $C_8H_{10}O_4N_2AsNa$: N, 9.46 per cent; As, 25.32 per cent.
Found: N, 9.52 per cent; As, 25.08 per cent.

The potassium and ammonium salts were prepared in the same way as the sodium salt and form thin, glistening, hexagonal, microscopic platelets. On adding a calcium chloride solution to a solution of the sodium salt the calcium salt gradually separates as microscopic wedge-shaped prisms, containing no water of crystallization. Magnesia mixture causes no precipitate in the cold, but on warming the magnesium salt separates as a microcrystalline powder. Heavy

metal salts give immediate precipitates, the silver salt forming aggregates of thin, microscopic needles.

N-Phenylglycineamide-*p*-arsonic acid was also prepared as follows from *N*-phenylglycine methyl ester-*p*-arsonic acid by the action of ammonia.

N-(Phenyl-4-Arsonic Acid)-Glycine Methyl Ester.—40 gm. of *N*-phenylglycine-*p*-arsonic acid[3] were treated with 120 gm. of dry methyl alcohol and 4 gm. of concentrated sulfuric acid. The mixture was boiled under a reflux condenser for 2 hours. The ester separated on cooling and scratching, the precipitation being completed by the addition of water. The filtered, washed, and dried product weighed 38 gm. It can be recrystallized from hot water or hot 95 per cent alcohol, separating from the former as microscopic needles and thin plates. It is very sparingly soluble in cold water, cold alcohol, or boiling acetone, and is fairly easily soluble in methyl alcohol, especially on warming. When rapidly heated it softens and darkens above 200°C. and decomposes at about 285°.

0.1560 gm. of substance; 7.0 cc. of N (21.0°C., 747 mm.).
0.3135 gm. of substance; 0.1665 gm. of $Mg_2As_2O_7$.
Calculated for $C_9H_{12}O_5NAs$: N, 4.84 per cent; As, 25.94 per cent.
Found: N, 5.12 per cent; As, 25.63 per cent.

The ester was converted into the amide as follows: 10 gm. of the ester were slowly added, with stirring, to 30 cc. of well chilled, concentrated ammonia. At first a thick paste of the ammonium salt of the ester was formed, but on allowing the mixture to rise to room temperature the reaction proceeded with formation of a clear solution. After 24 hours the excess of ammonia was removed, preferably *in vacuo*. On diluting with water, filtering, and acidifying with acetic acid, *N*-phenylglycineamide-*p*-arsonic acid separated in characteristic form. This was purified as described above and was identical in every way with the product obtained by the direct method. The yield was 80 per cent of the theory.

0.1297 gm. of substance; 11.5 cc. of N (19.5°C., 742 mm.).
Calculated for $C_8H_{11}O_4N_2As$: N, 10.22 per cent.
Found: N, 10.11 per cent.

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[3] German Patent, No. 204,664.