ON 3-AMINO-4-HYDROXYARSENOPHENYL-4'-GLYCINE AND ITS *N*-METHYLENESULPHINATE AND *N*-METHYLENESULPHONATE DERIVATIVES.*

BY MERRILL C. HART AND WILBUR B. PAYNE.

The chemotherapeutic theory of Ehrlich, which is based on Witt's theory of dyeing, lays stress upon the importance of so-called anchoring groups, which causes the drug to selectively attach itself to certain cells or organisms. Upon the organism to which the drug is thus selectively attached the arseno group (pharmacophore) then exerts an efficient therapeutic activity.

As his work progressed, it became evident that the activity of arseno compounds was due not to specificity but to the formation within the body of oxidation products which had very marked trypanocidal activity. It could be demonstrated *in vitro* that arsenoxides exert this therapeutic effect at great dilutions. These substances are, however, toxic to the host. The arsonic acids are of little therapeutic value and likewise they are correspondingly not so toxic.

The important work of Voegtlin and his associates has demonstrated the mechanism by reason of which arseno compounds combine lowered toxicity to the host and trypanocidal action on the infection. Arseno compounds *per se* are slightly toxic, but in the blood stream they are oxidized through the arsenoxide (trivalent stage) to the arsonic acid (pentavalent) stage and excreted. An arseno compound, to be of value, must not explosively oxidize and afford a concentration of arsenoxide which will prove toxic to the host, nor yet must it oxidize so slowly that the concentration of arsenoxide never becomes effectively trypanocidal. The dosage used must be based on these considerations. Also the rate of excretion of both original drug and its alteration products while the above is taking place has an important bearing. As a guide in this problem, it is possible to determine experimentally the maximum tolerated dose and the minimum effective dose, and a drug which can be borne in large doses and which is oxidized to a sufficient concentration of arsenoxide to be effective in small doses ought to prove valuable.

One can readily see how far this is removed from Ehrlich's pioneer chemophore conception. It was his opinion that the arsphenamine molecule, with ortho amino phenol groups, possessed this specific property to the highest degree. If this is true Voegtlin would ascribe the specific properties, not to the groups, but to the physical properties with which these groups had endowed the molecule in which they are present.

This particular molecule will have certain solubilities, rates of diffusion, etc. These in turn will markedly influence the rate of oxidation, path of excretion, and tendency to deposition in various parts of the body. Thus in a very indirect manner is the chemotherapeutic effect attained.

The determination of the minimum effective dose experimentally is interesting and no doubt has great significance as to the clearing up of primary lesions, but these experimental infections are different from clinical syphilis. Any sample of neoarsphenamine will clear up the primary lesions, but of the picture beyond this we have nothing but clinical trial to rely on. It may well be that certain arseno

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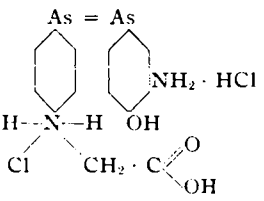
compounds which respond poorly to the experimental requirements as to minimum effective does may prove valuable.

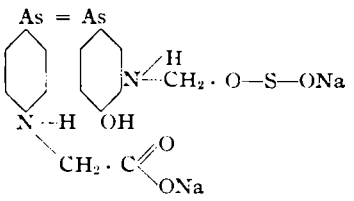
A large number of arseno derivatives have, of course, been prepared. Many of them have not been thoroughly studied. The description in the literature frequently betrays the fact that they have not been analyzed or prepared in such a manner as to warrant physiological study. Thus, 3-amino-4-hydroxyarsenophenyl-4′-glycine is described as red and must have been highly oxidized.

In arsphenamine the influence of the amino group is predominant. It has been a matter of great interest to observe the properties of arseno compounds in which this characteristic is altered and a more acidic influence given predominance. This has been done by double substitution directly (*e. g.*, diglycine derivative of arsphenamine[1]) or by the reduction of a substituted arsonic acid such as Ehrlich's arsenophenylglycine, for which Voegtlin[2] found a minimum lethal dose of 160 mg./Kg. and an effective dose of 10 mg./Kg. It appears that the absence of the phenol grouping has slightly impaired its efficiency.

We have been interested in the following arseno compounds.



I.



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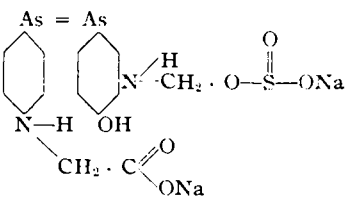

III.

If we consider (I) to be analogous to arsphenamine, (II) and (III) are comparable to neoarsphenamine and sulpharsphenamine, respectively.

The 3-amino-4-hydroxyarsenophenyl-4′-glycine (I) represents a compound that is very interesting from the theoretical standpoint to compare directly with arsphenamine. One-half of the molecule is identical with arsphenamine and the other half is deficient in the anchoring groups considered by Ehrlich to be necessary for the highest type of therapeutic efficiency and also contains the solubilizing group CH_2COOH. According to the theory of Ehrlich this compound would be decidedly inferior to arsphenamine therapeutically. If we consider the theory of Voegtlin that these groups merely act to modify the physical properties of the compound and these in turn influence the toxicity and the parasiticidal actively it is possible to conceive that this type of compound might be just as effective as arsphenamine. The laboratory findings tended to show that this compound was just as active therapeutically as arsphenamine and that the effect of the solubilizing

[1] D. R. P. 250,745.

[2] *J. Pharmacolog.*, 16, 6, 449, 1921.

group CH_2COOH was to lower the toxicity slightly and this was paralleled by a corresponding decrease in activity, the therapeutic ratio indicating this compound to be in the same class as arsphenamine.

At the present time more interest appears in the preparation of water-soluble condensation products which will prove somewhat less bothersome to handle. All these, however, appear to alter the arseno compounds in a similar manner, the characteristic increased solubility being the chief influence. As we go from arsphenamine to neoarsphenamine and sulpharsphenamine we produce compounds with a lower toxicity, but this decrease in toxicity is also accompanied by a corresponding decrease in their activity trypanocidally. We were interested in the question whether the introduction of these same groups would have the same effect in decreasing toxicity and trypanocidal activity when introduced in a molecule differing from arsphenamine in that it already had a solubilizing group (CH_2COOH) on one-half of the molecule, the other half being identical with arsphenamine (Compounds II and III).

These substances are readily prepared, and afford by comparison a measure for observing the influence of mono- and di-substitution by various groups on the same basic arseno compound. We were particularly interested in the different effects produced by substitution with the sulphur esters as distinct from the glycine substitutions. These results will be found in the summary.

The compound (I) 3-amino-4-hydroxyarsenophenyl-4′-glycine can be prepared by two methods. The 3-amino-4-hydroxyphenyl arsine reacts[1] with 4-glycinephenyl arsenious oxide (or chloride) to form the desired product. A much simpler method for its production is the reduction[2] of equimolecular mixtures of phenylglycine-*p*-arsenious chloride hydrochloride and 3-amine-4-hydroxyphenylarsenious oxide with sodium hydrosulfite.

EXPERIMENTAL.

The Action of Chloroacetic Acid upon 3-Amino-4-hydroxyphenylarsinic Acid.—3-Amino-4-hydroxyphenylarsinic acid 2.33 Gm. (1 mol.), chloroacetic acid 1.89 Gm. (2 mols.), sodium carbonate 2.1 Gm. (2 mol.), and potassium iodide, 3.2 Gm. (2 mols.), were heated under a reflux for $1^1/_2$ hours. A further quantity of sodium carbonate (0.5 Gm.) was required to render the solution yellow to methyl red, and the heating was continued two hours longer. The solution (100 cc) concentrated to 25 cc and acidified with acetic acid yielded no precipitate. It was concentrated to 10 cc, acidified with hydrochloric acid and upon standing 0.75 Gm. material crystallized (m. p. 255°). It was recrystallized from 5 cc of hot water and analyzed after drying it at 130° in a vacuum.

Subs., 0.1111 Gm., 6.79 cc, 0.1 *N* $Na_2S_2O_3$ (Lehmann).
Subs., 0.1723, 0.0824 Gm. $Mg_2As_2O_7$.
Calc. for $C_{10}H_{10}O_7NAs$, As, 22.7. Found, As, 22.92, 23.08.

The substance is probably an anhydride of the diglycine with both acetic groups on the nitrogen.

3-Amino-4-hydroxyphenylarsinic Acid.—This compound was prepared from 3-nitro-4-hydroxyphenylarsinic acid by reduction with ferrous hydroxide.[3] This

[1] D. R. P. 254,187.
[2] D. R. P. 251,104.
[3] W. A. Jacobs and M. Heidelberger, *J. Am. Chem. Soc.*, 40, 1580, 1918.

was purified by dissolving it in the calculated quantity of normal sodium hydroxide, treating it with animal charcoal, filtering hot, and precipitating with acetic acid. By this means, the 3-amino-4-hydroxyphenylarsinic acid was obtained as a white crystalline compound entirely soluble in dilute hydrochloric acid. It was dried to constant weight *in vacuo* and analyzed.

Subs., 0.2500, 0.2500, 21.08, 21.08 cc, 0.1 *N* $Na_2S_2O_3$ (Lehmann).
Calc. for $C_6H_8O_4NAs$, As, 32.19. Found As, 31.60, 31.60.

3-Amino-4-hydroxyphenylarsenious Oxide.—This substance was prepared by the method of Ehrlich and Bertheim[1] by the reduction of 3-amino-4-hydroxyphenylarsinic acid by sulphur dioxide. By this method preparations containing 40 to 60 per cent. of the oxide mixed with salt were produced. These were analyzed for arsenic and also by titration with iodine. It was found that the titration with iodine tended to give slightly high results and the figure for arsenic was used in all the calculations.

Phenylglycine-p-arsinic Acid.—This was prepared by the method outlined by Morgan.[2] The crude product was washed with dilute hydrochloric acid and crystallized from hot water. From 100 grams of arsanilic acid, 94 grams of dry crystalline acid were obtained. This was entirely soluble in dilute sodium bicarbonate solution.

Phenylglycine-p-arsenious Chloride Hydrochloride.[3]—Forty grams of the phenylglycine-*p*-arsinic acid were dissolved in 200 cc of hydrochloric acid (sp. gr. 1.19) to which 1 Gm. of potassium iodide was added. This solution was cooled in salt ice-bath to —10° C. and saturated with sulphur dioxide. A crystalline magma formed which was filtered off and washed with acetic acid and then with ether. It was dried to constant weight *in vacuo* over sodium hydroxide. Yield, 32.2 Gm.

Subs., 0.2000, 11.61 cc, 0.1 *N* $Na_2S_2O_3$ (Lehmann).
Calc. for $C_8H_9O_2NAsCl_3$, As, 22.55. Found, 21.76.

3-Amino-4-hydroxyarsenophenyl-4'-glycine.—We attempted to prepare this compound by reduction with hydrosulphite of equimolecular mixtures of the two different arsinic acids. Varying conditions as to time, temperature and acidity were employed, but in all cases mixtures were obtained usually with arsphenamine predominating. It thus appears that this class of compounds are not necessarily produced by this process. A review of the patent literature indicates this fact, but, on the contrary, one gathers from the current literature that this is a method of general application.

When 3-amino-4-hydroxyarsenophenyl-4'-glycine is prepared in pure condition in such a manner to prevent oxidation from exposure to the air it forms a bright yellow amorphous substance which dissolves in dilute sodium bicarbonate solutions and in dilute hydrochloric acid. The corresponding dihydrochloride when prepared by the Ehrlich process tends to hydrolyze so that dilute aqueous solution may appear very slightly turbid. Such a solution (1–100) will clear up when a few drops of tenth-normal hydrochloric acid is added. Such a solution when gradually

[1] *Ber.*, 45, 1, 756, 1912.
[2] G. T. Morgan, "Organic Compounds of Arsenic and Antimony," p. 165.
[3] D. R. P. 251,104.

treated with dilute bicarbonate at first precipitates the free arseno compound which redissolves with more of the bicarbonate to a brilliantly clear solution. It can be precipitated with acetic acid. The presence of free carbonic acid tends to retard a second solution with bicarbonate, and the bicarbonate solution can be precipitated by saturation with carbon dioxide.

The solubility in dilute bicarbonate and hydrochloric acid is the best criterion for the purity of this compound, as the arsenic to nitrogen or arsenic to chlorine ratios signify nothing as to its purity.

In order to prepare this arseno compound in a condition of sufficient purity to satisfy the above-mentioned solubility requirements we proceeded as follows: 24.96 Gm. of phenylglycine-*p*-arsenious chloride hydrochloride and 14.94 Gm. (37.58 Gm. of 40% material) of 3-amino-4-hydroxyphenylarsenious oxide were dissolved in 120 cc of methyl alcohol. This solution was poured into 300 cc of normal sodium hydroxide in a reduction cell[1] and diluted with 1200 cc of water. To this 120 Gm. of pure sodium hydrosulphite are added. The reduction takes place almost immediately at room temperature with the production of a heavy light yellow precipitate. The reaction was allowed to run for 15 minutes. At the end of this time, the base was filtered off, washed and dried and the hydrochloride prepared and ampouled using similar apparatus and technique to prevent oxidation used by Heyl and Miller for the preparation of pure arsphenamine hydrochloride. Seventy-five cc of 2 *N* methyl alcoholic hydrochloric acid diluted to 225 cc with absolute methyl alcohol were used for the solution of the base. For the precipitation of the hydrochloride, 2 liters of dry ether were sufficient. This gave 26 Gm. (72% theory) of the dihydrochloride. It was an amorphous light yellow powder.

Due to the colloidal nature of this material, the base filtered and dried very slowly even at pressures of 2–3 mm. of mercury. For the preparation of smaller amounts of this substance, it was found more convenient to centrifuge the base, wash it thrice with water, twice with absolute alcohol and once with ether. Because of the slight solubility of the base in alcohol, the yields (60–65% theory) by this method were not as good as the method given above.

One preparation was dried to constant weight *in vacuo* at 75° C. and analyzed. Volatile matter = 7.04%.

Analyses: Subs., 0.2000, 0.2000: 16.38, 16.48 cc, 0.1 *N* $Na_2S_2O_3$ (Lehmann).
Subs., 0.2325, AgCl, 0.1480.
Calc. for $C_{14}H_{14}O_3N_2As_2.2HCl$, As, 31.18; Cl, 14.76. Found, As, 30.69, 30.88, Cl, 15.74.

This material contained small amounts of sulphur.

Analysis: Subs. 0.3605, $BaSO_4$, 0.0075. Found, S, 0.28%.

Toxicity by the Rat Method.—3-Amino-4-hydroxyarsenophenyl-4′-glycine is less toxic than the arsphenamine. This property falls midway between arsphenamine and the well-known neoarsphenamine. The substance presents marked colloidal properties, and is somewhat more difficultly soluble than arsphenamine. For injection two per cent. solutions were employed. The substance was first dissolved in fresh distilled water and then made alkaline with the calculated quantity of nor-

[1] Heyl and Miller, Jour. A. Ph. A., 11, 434, 1922.

mal sodium hydroxide (4 moles) and made up to the mark. The observational period was 48 hours.

TABLE I.

TOXICITY OF SODIUM-3-AMINO-4-HYDROXYARSENOPHENYL-4'-GLYCINE.

Expt.	As, %.	Cl, %.	Doses in mg./Kg. 150.	175.	200.	225.	250.
54	28.54	14.64	+ + + + +	— — — — —			
60	29.68	13.03	+ + + + —	+ + — — —	+ + — — —		
65	27.78	14.93		+ — — — —	+ + + + —		
65	27.78	14.93	— — + + +	+ + + + +	+ + + + +	— — — — —	— — —
87	29.52	15.36	+ + + + —	+ + + — —			

+ Lived. — Died.

The maximum tolerated dose ranged from 150 to 200 mg./Kg. 78% of the deaths took place within the first 24 hours. It is well known that the toxicity of an arsphenamine solution can be decreased by standing, etc. 3-Amino-4-hydroxyarsenophenyl-4'-glycine shows this property to such an extent that we have observed animals injected with smaller doses to succumb, while injections made later proved less toxic. In Expt. 65 above, this is well illustrated.

Trypanocidal Activity of Sodium-3-amino-4-hydroxyarsenophenyl-4'-glycine.—Some observations were made to determine the minimum effective dose required to sterilize rats which had previously been infected with *Trypanosoma equiperdum.*[1] The methods employed by Voegtlin and Miller[2] were used. The therapeutic ratio (m. l. d./m. e. d.) was determined on two samples having a minimum lethal dose of 175 mg./Kg. The experimental infection was fairly uniform and varied from 150,000 to 250,000 per cmm. of blood. In order to sterilize in 24 hours, 5.2 to 9.2 mg./Kg. were necessary. If the observations as to sterility were made in 21 days the average dose required was 11.8 mg./Kg. For the 24-hour period the therapeutic ratio is from 19 to 33 and for the 21-day period, 15. Voegtlin and Miller found the therapeutic ratio of arsphenamine to vary between 13.2 and 26 and for neoarsphenamine it averaged 24.3 so that the safety of this drug is of the same order.[3]

Sodium-3-amino-4-hydroxyarsenophenyl-4'-glycine-N-methylenesulphinate.—We were interested in blocking both of the amino groups and to observe the effect of this on the toxicity and the parasiticidal activity of the drug. 3-Amino-4-hydroxyarsenophenyl-4'-glycine was therefore condensed with sodium formaldehyde sulphoxylate using the apparatus to prevent oxidation described by Heyl and Miller,[4] for the preparation of pure neoarsphenamine.

The glycine derivative (8 Gm.) was dissolved in 50 cc of absolute methyl alcohol. Nine cc of 50% formaldehyde sulphoxylate were used and the reaction ran for 3 minutes at 21° C. At the end of this time 12 cc of water and 23.5 cc of 10% sodium carbonate solution were added. The filtered solution was precipitated by running it into 400 cc of absolute alcohol. Made by this procedure the sodium 3-amino-

[1] The strain of *Trypanosoma equiperdum* used in this work was kindly furnished by Dr. Dyer of the Hygienic Laboratory, U. S. P. H. S.

[2] *Public Health Reports*, 37, 27, 1627, 1922.

[3] Clinical study of 3-amino-4-hydroxyarsenophenyl-4'-glycinedihydrochloride has been undertaken by Dr. A. D. Hirschfelder of the Pharmacology Department. University of Minnesota.

[4] *Loc. cit.*

4-hydroxyarsenophenyl-4′-glycine-*N*-methylenesulphinate formed a bright yellow, extremely soluble amorphous powder. It was dried in the usual way *in vacuo* over P_2O_5 and ampouled. 80% yields were obtained by this method. The dried powder averaged 25.66% arsenic content.

Determination of the Maximum Tolerated Dose.—For the determination of this value we used the same procedure given by us[1] for the determination of the maximum tolerated dose of neoarsphenamine. 3.088 Gm. of the hydrochloride of 3-amino-4-hydroxyarsenophenyl-4′-glycine (As, 28.5%) were dissolved in 15 cc of methyl alcohol. The condensation was made with 3.60 cc of 50% formaldehyde sulphoxylate solution (2 mols.). The reaction was complete in 3 minutes at 21° C. The yellow precipitated neo-compound is dissolved in 9.34 cc of 10% sodium carbonate forming the di-sodium salt. The solution after the removal of the alcohol in a vacuum is made up quantitatively to 110 cc. This solution represents 4% solution of the drug containing 20% arsenic. This arsenic content was selected to make the results directly comparable with neoarsphenamine.

Sodium 3-amino-hydroxyarsenophenyl-4′-glycine-*N*-methylene sulphinate is a more delicate chemical than neoarsphenamine. When the time of reaction is increased and the temperature of the condensation raised to 29° C. discoloration of the solution and increased toxicity result. (See Expt. 67, Table II.)

TABLE II.

TOXICITY OF SODIUM-3-AMINO-4-HYDROXYARSENOPHENYL-4′-GLYCINE-*N*-METHYLENESULPHINATE.

Expt.	"Glycine" used.	Temp.	Time.	As, %.	Doses in mg./Kg. 250.	300.	350.	400.	450.
67	54	29°	15 min.	20.3	+++++	++−−−		−−−−−	
73	60	25°	5 min.	20.33	+++++	+++++−	+++++−		
83	60	21°	3 min.	19.72			+++++	+++−−	−−−−−
85	65	21°	3 min.	18.98			+++++	+++−−	−−−−−

\+ Lived. − Died.

The rats used for the above tests showed a tolerance of 480 mg./Kg. for neoarsphenamine (20% arsenic). Similar to high test neoarsphenamine a majority of the deaths (86%) took place in the first 24 hours. This "neo-glycine" as far as toxicity is concerned is comparable to neoarsphenamine having a maximum tolerated dose of from 350 to 400 mg./Kg. (20% As).

Trypanocidal Activity of Sodium-3-amino-4-hydroxyarsenophenyl-4′-glycine-N-methylenesulphinate.—This was determined in a similar manner to that of the parent glycine derivative. The infection varied from 100,000 to 300,000 to the cmm. of blood. For sterilization in 24 hours dosages of 19 to 28 mg./Kg. were required. For the 21-day periods a 28 mg./Kg. dose was sufficient. The m. l. d. was 450 mg./Kg. at 20% arsenic. The therapeutic ratio found therefore varied from 16 to 24 for the 24-hour period and was 16 for the 21-day period.

These preparations were analyzed to observe the extent of the condensation. Twenty-five cc of the 4% solution were diluted to 60 cc with water and precipitated in the cold with 6 cc of normal hydrochloric acid. The slightly yellow precipitated "neo-acid" was centrifuged off and washed thrice with ice-cold water containing 2 cc of normal hydrochloric acid and then made up to 50 cc quantitatively with

[1] M. C. Hart and W. B. Payne, *J. Am. Chem. Soc.*, 44, 5, 1150, 1922.

water containing 4 cc of normal sodium hydroxide. Aliquots of this solution were used for the determination of arsenic, total sulphur, and sulphur condensed on the amino group ($NHCH_2OSOH$) using the same methods reported by us in a previous paper.[1] An average recovery of 98 per cent. of the arsenic in the precipitated and washed "neo-acid" showed the absence of any appreciable amount of the unchanged parent glycine in this product. The calculations are reported in terms of atomic ratios. Theory for the pure mono-methylenesulphinic acid derivative requires 2 arsenic to 1 sulphur. The findings are as follows:

TABLE III.

SULPHUR DISTRIBUTION IN 3-AMINO-4-HYDROXYARSENOPHENYL-4'-GLYCINE-*N*-METHYLENESULPHINATE.

Expt.	Temp.	Time.	Total S.	"Amino" S.	Nuclear S.
67	29° C.	15 min.	1.00	0.95	0.05
73	25° C.	5 min.	0.94	0.90	0.04
83	21° C.	3 min.	0.93	0.90	0.03
85	21° C.	3 min.	0.95	0.92	0.03

The reaction is practically complete in 3 minutes at 21° C. A higher temperature and a longer time (No. 67) gives a compound closer to the theoretical As to S ratio but darker in color and more toxic (see Table II).

Sodium-3-amino-4-hydroxyarsenophenyl-4'-glycine-N-methylenesulphonate.—It was thought that this derivative might have more satisfactory properties than the corresponding methylenesulphinate described above. Voegtlin and Smith[2] have described the di-methylenesulphonate of arsphenamine (sulpharsphenamine).

The introduction of the methylenesulphonate group in this case produces a compound that can be injected subcutaneously and which is quite stable when exposed in solution to the air.

The same precautions used in the preparation of all of these compounds to prevent oxidation from the air were also used in the preparation of this derivative. To 3-amino-4-hydroxyarsenophenyl-4'-glycine dihydrochloride 4 Gm. (27.8% As) dissolved in 6 cc of alcohol and 56 cc of water are added 0.66 cc of $33^1/_3\%$[3] formalin solution (1 mol.). After one minute 5.04 cc of 30% sodium hydrogen sulphite solution (2 mols.) were added. An immediate thick yellow precipitate was formed that slowly went into solution. At the end of 7 minutes 8 cc of 10% sodium carbonate solution were added. After 16 minutes it was filtered out of contact with air and precipitated by running it into 320 cc of absolute alcohol. The yellow granular precipitate was centrifuged off and washed twice with 150 cc of absolute alcohol. It was dried to constant weight *in vacuo* over sodium hydroxide and phosphoric anhydride. A yield of 4.08 Gm. was obtained. This was ampouled up and sealed in the presence of carbon dioxide in the usual manner. This compound formed a golden yellow amorphous powder averaging 23.8% arsenic. Solutions of it did not darken on exposure to the air in contradistinction to neoarsphenamine. Similar to Voegtlin and Smith's sulpharsphenamine this

[1] M. C. Hart and W. P. Payne, *Loc. cit.*

[2] Voegtlin and Smith, *J. Am. Chem. Soc.*, 44, 2574, 1922; also D. R. P. 249,726.

[3] The strength of the formalin and bisulphite solutions were determined by analysis.

compound did not reduce indigo carmine[1] solution showing the absence of the sulphinic acid grouping. Upon treating this with zinc and acetic acid the filtrate will reduce the indigo carmine solution due to the formation of the CH_2OSONa grouping.

One of these preparations was used for the preparation and analysis of the free acid. Two grams of the di-sodium salt of the 3-amino 4-hydroxyarsenophenyl-4'-glycine-*N*-methylenesulphonate were taken up in 8 cc of water and precipitated with 30 cc of glacial acetic acid. The yellow precipitated acid was centrifuged off and washed thrice with 25 cc of 80% acetic acid and finally dried to constant weight *in vacuo* over sodium hydroxide and finally over phosphoric anhydride. The yield of the dried acid was 1.250 grams. This was dried to constant weight *in vacuo* at 100° C. and analyzed. Moisture = 12.95%.

Analysis: Subs., 0.2000, 0.2000: 16.28, 16.38 cc 0.1 *N* $Na_2S_2O_3$ (Lehmann).
Subs., 0.3000, 0.3000, $BaSO_4$, 0.1329, 0.1348.
Calc. for $C_{15}H_{16}N_2O_6As_2S$: As, 29.88, S, 6.37. Found As, 30.50, 30.69, S, 6.08, 6.17.

The above figures for arsenic and sulphur when calculated in terms of atomic ratios indicate that the condensation had occurred to the extent of 94%.

Toxicity and Trypanocidal Activity of Sodium-3-amino-4-hydroxyarsenophenyl-4'-glycine-N-methylenesulphonate.—Two runs of this compound were prepared and the toxicity and trypanocidal activity determined. The minimum lethal dose in 4% solution (24% arsenic) by the official method for neoarsphenamine varied from 250 to 300 mg./Kg. The rats used for these tests showed a tolerance of 480 mg./Kg. for neoarsphenamine (20% arsenic). On rats carrying an infection of 100,000 to 180,000 of Trypanosoma equiperdum per cmm. of blood the dose required to sterilize for 24 hours as well as for the 3-day period was 15.5 mg./Kg. The therapeutic ratio therefore varied from 16 to 20.

Solutions of this compound could also be injected subcutaneously. By this method the minimum lethal dose was 350 mg./Kg. Subcutaneously it was also very active trypanocidally. 31 mg./Kg. doses were sufficient to sterilize rats for the 24-hour period which had an infection of 150,000 to 250,000 per cmm. of the trypanosomes. For the 3-day period doses of 23 mg./Kg. were required. The therapeutic ratio by this method is 11 to 15.

Solutions of this compound were quite stable when exposed to the air. A solution which killed 2 out of 5 rats at 250 mg./Kg. when allowed to stand 24 hours in an open cylinder exposed to air killed none out of 5 rats at the same dosage.

TABLE IV.

TOXICITY OF SODIUM-3-AMINO-4-HYDROXYARSENOPHENYL-4'-GLYCINE-*N*-METHYLENESULPHONATE.

		Doses in mg./Kg.			
Expt.	Injection.	200.	250.	300.	350.
89	Intraven.	+++++	++−−−		−−−
90	Intraven.	+++++	+++−−	−−−	−−−
90	Intraven.[1]	+++++	+++++	++++−	+−−−−
89	Subcut.	+++++	+++−+−	++++−	−−−
90	Subcut.			++++−	+−−−−

[1] Solution allowed to stand exposed to the air in an open cylinder for 24 hours.

[1] Reinking, Dehnel and Labhardt, *Ber.*, 38, 1069, 1905.

At a dose of 300 mg./Kg. one out of 5 rats were killed showing an actual decrease in toxicity on standing.

SUMMARY.

1. The following unsymmetrical arseno compounds have been prepared and studied with the following results:

(*a*) 3-Amino-4-hydroxyarsenophenyl-4′-glycine for which the maximum tolerated dose was found to be 150 to 200 mg./Kg. with a therapeutic index of 19 to 33 for the 24-hour period and 15 for the 21-day period.

(*b*) 3 - Amino - 4 - hydroxyarsenophenyl - 4′ - glycine - *N* - methylenesulphinate for which the maximum tolerated dose was found to be 350 to 400 mg./Kg. (20% As) and the therapeutic index 16 to 24 for the 24-hour period, and 16 for the 21-day period.

(*c*) 3 - Amino - 4 - hydroxyarsenophenyl - 4′ - glycine - *N* - methylenesulphonate for which the minimum lethal dose (intravenously) was 250 to 300 mg./Kg. and subcutaneously was 350 mg./Kg. (24% As). The therapeutic ratio of this intravenously varied from 16 to 20 for both the 24-hour and the 3-day period and subcutaneously it was 11 for the 24-hour period and 15 for the 3-day period. This compound is also very stable in solution and becomes less toxic on standing exposed to the air for 24 hours.

2. The introduction of the methylenesulphinate group into 3-amino-4-hydroxyarsenophenyl-4′-glycine reduces considerably the toxicity of the compound. The activity of the compound trypanocidally is also correspondingly reduced. The advantage in the introduction of this group into the molecule in this case is the production of a water-soluble derivative that is easier to handle clinically.

3. The introduction of the methylenesulphonate group into the molecule of the 3-amino-4-hydroxyarsenophenyl-4′-glycine results in a slighter diminution of the toxicity than the methylenesulphinate group. However, the activity is correspondingly slightly reduced. The great advantage though in the introduction of this group is the production of a water-soluble derivative that is stable when exposed to the air and which also can be injected subcutaneously.

4. The effect of the introduction of the methylenesulphinate and the methylenesulphonate group into the 3-amino-4-hydroxyarsenophenyl-4′-glycine on therapeutic activity (m. l. d./m. c. d.) parallels the effect of the same groups when introduced into arsphenamine.

5. The series of compounds described above are deficient in some of the essential anchoring groups considered by Ehrlich to be necessary for the highest type of therapeutic efficient which he claims to have reached in arsphenamine. If this is true these compounds should be distinctly inferior to the corresponding ones in the arsphenamine series (arsphenamine, neoarsphenamine, and sulpharsphenamine). The laboratory tests, however, did not substantiate the theory of Ehrlich but showed that these compounds were just as effective, therapeutically, as the corresponding ones in the arsphenamine series.

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RESEARCH LABORATORIES
OF THE UPJOHN COMPANY,
KALAMAZOO, MICH.