Another method used was to dissolve the phlobaphene in alcohol. From its alcoholic solution the phlobaphene could be precipitated by the addition of slightly acidulated water.

The phlobaphenes so obtained were first dried in an oven at 70° C. and then kept in desiccators until their weight remained constant. The following figures representing the results of some of the combustions carried out show how widely the various phlobaphenes differ.

	%	%	%
Krameria	C 62	H 5	O 33
Tormentilla	C 59	H 5	O 36
Bistorta	C 57	H 7.8	O 38.2
Kola Nuts	C 56.7	H 5	O 38.3

The phlobaphenes are the end products of a large number of natural tannins. They are the last step in a long chain of transformations, starting with simple crystallizable substances and ending with an amorphous complicated condensation product.

In concluding it might be interesting to mention an economic value, if only an indirect one of certain phlobaphenes. The beautifully attractive brown color of chocolate is in great measure due to a phlobaphene derived from a tannin originally present in the fresh cacao seed. We know that in the fresh cacao seed an alkaloid-tannoglucoside is present which during the process of fermentation to which the seeds are subjected is broken up into its component parts. The tannin is oxidized by oxidases present to cacao-red. The oxidation is progressive, the end product being the brown colored phlobaphene.

ON 3-AMINO-4:4′-DIHYDROXYARSENOBENZENE AND ITS *N*-METHYLENESULPHINATE AND *N*-METHYLENESULPHONATE DERIVATIVES.*,†

BY MERRILL C. HART AND WILBUR B. PAYNE.

For the production of unsymmetrical arseno compounds three general methods are given in the literature. According to Bertheim[1] the reduction of equimolecular mixtures of two different arsinic acids or oxides, or a mixture of an acid with an oxide leads only to the production of the unsymmetrical derivative and not to a mixture of the three possible products. Limiting the reduction agent used to sodium hydrosulphite we find Fargher[2] describing the unsymmetrical compound 3′-amino-4′-hydroxy-1:3-diazole-5:1′-arsenobenzene by reduction of the acids. Christiansen[3] also produces by this method 3-amino-4:4′-dihydroxyarsenobenzene and 3-5-3′-triamino-4:4′-dihydroxyarsenobenzene. To test the purity of these compounds Christiansen relied on the solubility tests and the percentage of arsenic.

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* Contribution from the Laboratories of The Upjohn Company.
† Received for Publication June 19, 1923.

[1] *Chem. Zeit.*, 38, 756, 1914.
[2] *J. Chem. Soc.*, 117, 866, 1920.
[3] *J. Am. Chem. Soc.*, 43, 2202, 1921.

These unsymmetrical compounds are also said to be readily produced[1] by the condensation of arsines[2] with arsenoxides or chloroarsines. This reaction has also been used to prepare the mixed antimony and other complex derivatives.[3]

$$RAsH_2 + OAsR' \longrightarrow RAs = AsR' + H_2O$$
$$RAsH_2 + Cl_2AsR' \longrightarrow RAs = AsR' + 2HCl$$

By this method the following unsymmetrical arseno derivatives have been prepared; 4-amino-4′-hydroxyarsenobenzene, 3-amino-4-hydroxyarsenophenyl-4′-glycine and 3-amino-4-hydroxyarsenobenzene.

The third method for the production of these compounds is by the rearrangement of two symmetrical compounds to form the unsymmetrical. Karrer[4] has

$$RAs = AsR + R'As = AsR' \longrightarrow 2RAs = AsR'$$

observed this only in the case of two compounds basing his conclusion on a change in solubilities when the two symmetrical compounds were heated together at 70° to 80°.

From the examination of the patent literature we find that when mixtures of two arsinic acids are reduced, stannous chloride in the presence of strong hydrochloric acid is used. By this method 3:4′-diamino-4-hydroxyarsenobenzene[5] is produced. However where the arsenoxides were used sodium hydrosulphite was the reducing agent employed. By this means were produced[6] 3-amino-4-hydroxyarsenophenyl-4′-glycine, 3-amino-4:4′-dihydroxy-3′-5′-diaminoarsenobenzene, 3-amino-4-hydroxyarsenobenzene, 3-amino-4-hydroxyarsenobenzene, 3-amino-4-hydroxyarsenomethane,[7] and 3′:5′-dichloro-3-amino-4:4′-dihydroxyarsenobenzene.

We have found in the several cases studied that reduction of an equimolecular mixture of two arsinic acids does not uniformly give the unsymmetrical compound and that the production of these is a much more difficult problem than one would gather from a cursory survey of the literature. The production of unsymmetrical derivatives by this method is based on two assumptions that up to this time have not been definitely proven. One of these is that the rate of reduction of the two different acids is the same so that the production of RAs= equals the production of R′As=, otherwise there would be a piling up of the symmetrical compound of the acid which was reduced the more rapidly for the radicle RAs= cannot exist but momentarily in the free condition. However if the time element is long enough for complete reduction with different rates of reduction and the consequent production of mixtures of the three possible products it would be possible to produce the unsymmetrical derivative if the two symmetrical compounds had the ability to rearrange spontaneously under the conditions employed to form the

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[1] Bertheim, "Handbuch. d. Org. Arsenoverb.," p. 162, 1913.
[2] Kahn, *Chem.-Ztg.*, 1099, 1912, D. R. P., 254,187, D. R. P., 251,571.
[3] D. R. P., 270,259.
[4] *Ber.*, 49, 1648, 1916.
[5] D. R. P., 251,104.
[6] D. R. P., 251,104.
[7] D. R. P., 253,226.

unsymmetrical. The rearrangement of compounds of this type has however only been studied in the two cases reported by Karrer.[1]

The observations of King[2] on unsymmetrical arseno compounds include the suggestion that the particular substance formed upon reduction is dependent upon the relative solubilities of the possible products so that in reducing a mixture of $RAsO_3H_2$ and $R'AsO_3H_2$ one would obtain RAs=AsR′ exclusively only if it were less soluble than RAs=AsR or R′As=AsR′. When aqueous hydrosulphite solutions are used for reduction these develop an acidity due to $NaHSO_3$ as the reduction proceeds so that the solution is neither basic nor yet strongly acidic and this will undoubtedly largely influence the nature of the products obtained.

For the preparation of this type of compound by reduction with hydrosulphite we found it advisable to use the oxides. These reduce almost immediately at room temperatures with the formation of the arseno compound. When the arsinic acids are used the reduction requires several hours at a temperature of 50° to 60°. It is our opinion that it is this rate of reduction that largely determines the nature of the end products.

The method of preparing these compounds by the reaction of an arsine with an arsenoxide is probably a general method for the preparation of these compounds. However the experimental difficulties of producing the arsine and condensing it with the arsenoxide before it is oxidized partially at least to the arseno stage make this method of little more than theoretical interest.

The question of the rearrangement of two symmetrical arseno compounds was investigated by us in the case of 4-4′-dihydroxyarsenobenzene and arsphenamine.

In the course of some of this work we produced 3-amino-4:4′-dihydroxyarsenobenzene. This represents arsphenamine minus one of its important anchoring groups according to Ehrlich. We were interested to see if this loss was reflected in its therapeutic efficiency.

EXPERIMENTAL.

The Preparation of Unsymmetrical Arseno Compounds by Reduction of Equimolecular Mixtures of the Arsinic Acids with Sodium Hydrosulphite.—Several attempts were made to prepare unsymmetrical compounds by this method. The experiments tried were selected so that the course of the reaction could be followed easily by solubility tests. Conditions analogous to those used for the production of arsphenamine were used. Supplementary experiments were also tried where the acids were reduced in the presence of sodium acetate and also where a preliminary reduction was carried out in the presence of strong acid with a trace of hydrogen iodide present, later making alkaline and reducing with sodium hydrosulphite.

4-Hydroxy-4-aminoarsenobenzene.—A number of attempts to prepare this by the reduction with sodium hydrosulphite of equimolecular mixtures of the two arsinic acids were tried. Mixtures of the three possible products were obtained in all cases usually with the symmetrical 4-4′-dihydroxyarsenobenzene predominating.

[1] *Loc. cit.*

[2] *J. Chem. Soc.*, 49, 1416, 1117, 1921.

4-Amino-arsenophenyl-4'-glycine.—Mixtures were also obtained in this case on reduction of equimolecular mixtures of phenylglycine-*p*-arsinic acid and arsanilic acid with sodium hydrosulphite under the conditions before given.

3-Amino-4:4'-dihydroxyarsenobenzene.—Christiansen[1] has described the production of this compound by the reduction of equimolecular mixtures of the two arsinic acids with sodium hydrosulphite using the conditions for the reduction used in the production of arsphenamine. For the determination of the purity of his product he relied on the solubilities and the arsenic determination. These, however, would not detect the presence of arsphenamine in this product as an impurity. We repeated the work of Christiansen and analyzed the product in the form of the hydrochloride for arsenic and nitrogen. These when expressed in terms of atomic ratios gave 2 arsenic to 1.08 nitrogen showing the admixture of appreciable amounts of arsphenamine in this product.

4-Hydroxyphenylarsenous Oxide.—The acid from which this was prepared was obtained by the method given by Jacobs and Heidelberger[2] by the fusion of phenol with arsenic acid and the isolation of the acid as the sodium salt. Fifty-seven grams of this sodium salt were reduced to the arsenoxide stage by the methods given in the patent.[3] This reduction must not be allowed to run for too long a period of time as sulphonation is liable to occur. From the 57 grams of the acid 21 grams of the dry arsenoxide[4] were obtained. As it was necessary to have the exact purity of this compound for subsequent work it was analyzed not only for arsenic but also by titration with 0.1 *N* iodine solution.

Subs., 0.2885; loss, 0.204 *in vacuo* at 100° over $CaCl_2$. Moisture, 7.06.
Subs., anhydrous, 0.2000, 0.2000; 21.48, 21.44 cc, 0.1 *N* $Na_2S_2O_3$ (Lehmann).
Subs., anhydrous, 0.2000, 0.2000; 21.55, 21.55 cc, 0.1 *N* iodine.
Calc. for $C_6H_5O_2As$: As, 40.76, 0.1 *N* iodine, 21.74.
Found: As, 40.27, 40.20, 0.1 *N* iodine, 21.58, 21.58.

3-Amino-4-hydroxyphenylarsenoxide.—This was prepared from 3-amino-4-hydroxyphenylarsinic acid by reduction[5] with sulphur dioxide. This was analyzed for arsenic and by titration with iodine to determine the purity. Products consisting of 40 to 60 per cent. of the arsenoxide mixed with salt were obtained.

3-Amino-4:4'-dihydroxyarsenobenzene.—The reduction of equimolecular mixtures of the two acids gave this product contaminated with appreciable quantities of arsphenamine. The reaction of 3-amino-4-hydroxyphenylarsine with 4-hydroxyphenylarsenious oxide can be used to prepare this compound but the experimental difficulties make this only of theoretical interest. The two symmetrical compounds put together in alkaline solution and held there for two hours at 55° to 60° C. did not rearrange to give this unsymmetrical derivative. We found that the reduction of equimolecular mixtures of 3-amino-4-hydroxyphenylarsenious oxide and 4-hydroxyphenylarsenious oxide gave the derivative desired quickly and in practically quantitative yields. One prerequisite for the success of this experiment

[1] *Loc. cit.*
[2] *J. Am. Chem. Soc.*, 41, 1446, 1919.
[3] D. R. P., 213,514.
[4] Care must be used in working with this as it is very irritating to the mucous membrane of the nose and throat.
[5] P. Ehrlich and A. Bertheim, *Ber.*, 45, 1, 756, 1912.

though was the exact quantitative determination of the purity of the oxides used.

For the production of 3-amino-4:4′-dihydroxyarsenobenzene in a state of purity we proceeded as follows: 16.400 grams (44.9% oxide) of the 3-amino-4-hydroxyphenylarsenious oxide and 7.400 grams (92.0% oxide) of the 4-hydroxyphenylarsenious oxide were dissolved in 60 cc. of methyl alcohol and 37.2 cc of *N* sodium hydroxide were added and the solution diluted with 600 cc of water in a reduction cell[1] and 60 grams of sodium hydrosulphite (90%) were added. An immediate thick yellow precipitate of the arseno compound resulted. The reaction was allowed to continue for 15 minutes at 20° C. At the end of this time it was filtered off, washed and dried in an atmosphere of carbon dioxide in the patent filter desiccator described by Heyl and Miller. The yield of the dry base was 13.1 grams. The hydrochloride was prepared from this by dissolving the base in 65 cc of absolute methyl alcohol containing 18.5 cc of 2 *N* methyl alcoholic hydrochloric acid, filtering in an atmosphere of nitrogen and precipitating by running it into one liter of dry chilled ether. The grayish colored hydrochloride was centrifuged off and washed with 400 cc of ether and dried *in vacuo* over phosphoric anhydride. The yield of the hydrochloride was 13.3 grams (93%). This formed an amorphous light gray powder containing 35.15% arsenic. This material was soluble in dilute sodium hydroxide and in dilute hydrochloric acid solution showing the absence of the symmetrical derivative, 4:4′-dihydroxyarsenobenzene. The material was ampouled up and sealed in the presence of carbon dioxide in the usual manner.

The product was analyzed for arsenic, nitrogen, and chlorine and these expressed in terms of atomic ratios showed the absence of any detectable amounts of the two possible symmetrical impurities.

Subs. 0.2000, 0.2000, 18.75 cc, 18.75 cc, 0.1 *N* $Na_2S_2O_3$ (Lehmann).
Subs. 0.3000, 0.3000, 14.18 cc, 14.31 cc, 0.05 *N* HCl (Kjeldahl).
Subs. 0.3000, 0.3000, AgCl, 0.1014, 0.1030.
Calc. for $C_{12}H_{12}O_2$ $NAs_2Cl.2H_2O$: As, 35.42, N, 3.30, Cl, 8.38.
Found: As, 35.15, 35.15, N, 3.31, 3.34, Cl, 8.37, 8.49.

Toxicity and Trypanocidal Activity of 3-Amino-4:4′-dihydroxyarsenobenzene.— The toxicity of this was determined using the method for arsphenamine. Two per cent. solutions were employed containing sufficient *N* sodium hydroxide (3 mols.) to form the di-phenolate (calculated from the percentage of arsenic). The observational period was two days. The maximum tolerated dose was 140 mg./Kg.

Some observations were made to determine the minimum effective dose required to sterilize rats which previously had been infected with *Trypanasoma equiperdum*. The methods employed by Voegtlin[2] and Miller were used. The therapeutic ratio was determined on a sample having a minimum lethal dose of 160 mg./Kg. The experimental infection was fairly uniform and varied from 135,000 to 150,000 per cmm. of blood. In order to sterilize in 24 hours 6.3 mg./Kg. doses were necessary. If the observations as to sterility were made in 3 days the

[1] Heyl and Miller, JOUR. A. PH. A., 11, 6, 431, 1922.

[2] *Public Health Reports*, 37, 27, 1627, 1922. The strain of *Trypanasoma equiperdum* used in this work was kindly furnished by Dr. R. E. Dyer, Assistant Director, Hygienic Laboratory, United States Public Health Service.

average dose required was the same. For the 24 hour period the therapeutic ratio was 25 and for the 3 day period the same.

3-Amino-4:4'-dihydroxyarsenobenzene-N-methylenesulphinate.—We were interested in the determination of the maximum tolerated dose of this derivative. We used for this the same procedure used by us for the determination of the maximum tolerated dose[1] of neoarsphenamine. 2.5144 grams of the hydrochloride of 3-amino-4:4'-dihydroxyarsenobenzene (35% As) were dissolved in 15 cc of methyl alcohol containing 1.47 cc of 2 *N* methyl alcoholic hydrochloric acid. The condensation was made with 3.60 cc of 50% formaldehyde sulphoxylate solution (2 mols.). The reaction was run for 15 minutes at 21° C. and then 6.24 cc of 10% sodium carbonate solution were added. The solution after the removal of most of the alcohol in a vacuum was made up quantitatively to 110 cc. This solution represents a 4% solution of the drug containing 20% arsenic.

Sodium 3-amino-4:4'-dihydroxyarsenobenzene-*N*-methylenesulphinate is a very unstable chemical, solutions of it decomposing very quickly. It had a maximum tolerated dose of 250 mg./Kg. Lethal doses produced immediate symptoms. The animals resisted the latter part of the injection and showed dyspnea followed quickly by convulsions and final collapse.

The free neo-acid from this solution was precipitated out, washed and the arsenic, total sulphur, and amino sulphur determined using the same methods[2] we used for neoarsphenamine. The loss in arsenic in the precipitated neo-acid was from 2 to 3 per cent., showing the absence of any appreciable amount of the unreacted upon arseno compound in this product.

The percentages of arsenic and sulphur expressed in terms of atomic ratios were 2 to 0.68 for the amino sulphur and 2 to 0.75 for the total sulphur. These analytical results indicate that the *N*-methylene sulphinic acid derivative first formed probably reacted with some of the unchanged arseno compound with the formation of a salt-like compound of the type $RNHCH_2OSONH_3R$ and the product was a mixture of this with the 3-amino-4:4'-dihydroxyarsenobenzene-*N*-methylenesulphinate. Binz and Marx[3] report this same type of reaction when aniline and orthotoluidin react with formaldehyde sulfoxylate.

This condensation was run at a higher temperature (30° C.) and for a longer time (30 minutes) but the arsenic to sulphur ratios showed the production of the same type of compound.

3-Amino-4:4'-dihydroxyarsenobenzene-N-methylenesulphonate.—It was thought that this derivative might have more satisfactory properties than the corresponding *N*-methylenesulphinate described above. The same technique used in the preparation of neoarsphenamine was used in the production of this compound. To 4 Gm. of 3-amino-4:4'-dihydroxyarsenobenzene hydrochloride (35.15% As) dissolved in 6 cc of methyl alcohol and 56 cc of water were added 0.86 cc (1 mol.) of a $33^1/_3$ per cent. solution of formalin.[4] At the expiration of one minute[5] 3.25 cc of a 30% sodium bisulphite solution (1 mol.) were then added. A thick copious yellow

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

[2] Hart and Payne, *Loc. cit.*

[3] *Ber.*, 43, 2344, 1910.

[4] The strength of the formalin and bisulphite solutions were determined exactly by analyses.

[5] Longer times or an excess of formalin result in the formation of a jelly.

precipitate formed immediately and then slowly went into solution as the reaction proceeded. At the end of 10 minutes another mole of the bisulphite was added. The solution after 20 minutes was filtered in an atmosphere of nitrogen and precipitated by running into 400 cc of absolute alcohol. The cream colored precipitate was centrifuged off and washed with 300 cc of alcohol and then dried to constant weight over P_2O_5. A yield of 4.04 grams were obtained, containing 28.63% arsenic.

The substance was analyzed in the following manner: 2 grams of the sodium salt of the 3-amino-4:4′-dihydroxyarsenobenzene-*N*-methylenesulphonate were dissolved in 10 cc of water and precipitated with 25 cc of glacial acetic acid. The yellow precipitated acid was centrifuged off and washed 4 times with 30 cc of 85% acetic acid. The acid was finally made up quantitatively to 50 cc as the sodium salt and aliquots of this were used for the arsenic, total sulphur, and amino sulphur determinations.

The results of these analyses expressed in terms of atomic ratios showed the presence of 2 arsenic to 0.60 sulphur for the amino value and 2 to 0.65 for the total sulphur value. These results indicate that this reaction is of the same type as the methylene sulphinate discussed above.

Toxicity and Trypanocidal Activity of Sodium 3-Amino-4:4′-dihydroxy-arsenobenzene-N-methylenesulphonate.—The minimum lethal dose in 4% solution (29% As) by the official method for neoarsphenamine was 150 mg./Kg. On rats carrying an infection of 200,000 to 250,000 of *Trypanasoma equiperdum* per cmm. of blood the dose required to sterilize in 24 hours was 13 mg./Kg. and for 3 days was 7.8 mg./Kg. The therapeutic ratios therefore were 12 and 20, respectively.

Solutions of this compound could also be injected subcutaneously. By this method the minimum lethal dose was 250 mg./Kg. Subcutaneously it was also active trypanocidally. A dose of 19 mg./Kg. was required to sterilize rats for the 24 hour period which had an infection of 200,000 to 250,000 per cmm. of the trypanasomes. For the 3 day period 19 mg. were also required. The therapeutic ratio by this method is 13.

Solutions of this compound were also quite stable when exposed to the air. A solution which killed 4 out of 5 rats at 150 mg./Kg. when allowed to stand 24 hours in an open cylinder killed 2 out of 5 at the same dosage.

SUMMARY.

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

(*a*) 3-Amino-4:4′-dihydroxyarsenobenzene for which the maximum tolerated dose was found to be 140 mg./Kg. with a therapeutic ratio of 25.

(*b*) 3-Amino-4:4′-dihydroxyarsenobenzene-*N*-methylenesulphinate for which the maximum tolerated dose at 20% arsenic was 250 mg./Kg.

(*c*) 3-Amino-4:4′-dihydroxyarsenobenzene-*N*-methylenesulphonate for which the minimum lethal dose intravenously was 150 mg./Kg. and subcutaneously 250 mg./Kg. The therapeutic ratios by these two methods were 12 to 20 and 13, respectively.

This compound is also stable and does not become more toxic on standing exposed to the air for 24 hours.

2. Reduction of molecular mixtures of the different arsenic acids with sodium hydrosulphite is not a general method for the production of unsymmetrical derivatives.

3. The loss of one of the essential anchoring groups, according to Ehrlich, from arsphenamine does not impair its therapeutic efficiency as far as these laboratory tests show.

In conclusion we wish to thank Dr. Frederick W. Heyl at whose suggestion this work was carried out.

KALAMAZOO, MICH.

ALOIN.

BY H. ENGELHARDT AND H. H. CROSBIE.

Aloin is defined in the United States Pharmacopœia as a pentoside or a mixture of pentosides, *i. e.*, compounds which resemble the glucosides and differ from them only in that one of the components is not a carbohydrate proper, but a five-atomic alcohol. Until about 20 years ago aloin was classed among the bitter principles, until Léger found that on prolonged hydrolysis with alcoholic hydrochloric acid it is split up into 1.8 dioxi-3 methylenoxianthraquinone and *d*-arabinose.

While introducing refinements in the process of manufacture, with the idea of greater purity, some difficulty was encountered with the solubility of aloin in water, which the U. S. P. states is 1 : 120. It was considered possible that in the manufacturing process of aloin under certain physical conditions (concentration, temperature and rate of crystallization) a product was obtained, which contained a larger amount of other less soluble pentosides. It was found that when shaking aloin with water in the proportion of 1 : 120, an apparently large amount of silky needles remained undissolved which made the solubility of the aloin not respond to the official requirement, *i. e.*, that not more than 1.5 per cent. of the aloin should be insoluble in the prescribed amount of water. The present U. S. P. Revision Committee proposed that the amount of water-insoluble matter be reduced to one per cent. and it was, therefore, interesting to find out whether or not aloin made by different reputable manufacturers would come up to this requirement. It was found that only one of these aloins out of four contained less than one per cent. of water-insoluble matter and this specimen had a pronounced odor of aloes which suggested that the mother-liquid had only insufficiently been removed. It was also found that a specially pure sample, which showed insufficient solubility, could be made to conform to U. S. P. requirements by the addition of 5% of powdered aloes, apparently a serious reflection on the test. And experiments showed that the solubility of aloin can be somewhat increased by not completely eliminating the mother liquid, but we believe that by doing so an aloin is obtained which is not completely devoid of the griping principles of the drug. To be frank, the insufficient solubility of the aloin was not due to the presence of other compounds (pentosides?) but to the temperature at which the solubility test had been carried out, because an aloin which when shaken with water in a