2. The ternary system, silver perchlorate-toluene-water has been studied from the ternary eutectic at —94° up to +91.75°. There exist seven quintuple points, each of which was determined, and twenty 4-phase equilibria.

3. The system shows, in addition to the solubility curves for the three solid phases (silver perchlorate, its hydrate and its compound with toluene), two binodal curves, one of which is submerged and does not reach any of the two-component axes at any temperature.

4. The intersection of the two binodal curves, at certain points which are not their plait points, gives rise to a 3-liquid system which is stable from —24.1° to above +90°.

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THE SULFUR CONTENT OF ARSPHENAMINE AND ITS RELATION TO THE MODE OF SYNTHESIS AND THE TOXICITY. V.[1]

BY W. G. CHRISTIANSEN, A. J. NORTON AND J. B. SHOHAN

Previous studies[2] in this Laboratory have shown that when 3-nitro-4-hydroxyphenylarsonic acid is reduced to arsphenamine base by means of sodium hydrosulfite as indicated in Reaction 1, the experimental conditions under which the nitro acid is reduced to the amino acid are of great importance in determining the toxicity and sulfur content of the reaction product.

$$\tag{1}$$
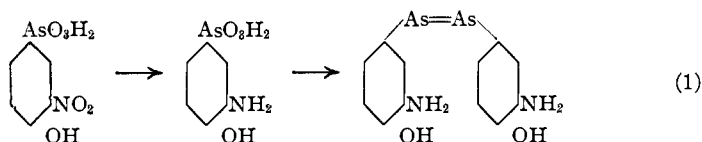
Evidence has also been reported[3] of the formation of a very soluble by-product during the reduction of the nitro group; it was hypothetically suggested[4] that sulfamic acids are formed and that the latter give rise to the sulfo-arseno compounds which are present as impurities in the arsphenamine obtained when the nitro group is reduced under the least favorable

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[1] This is a continuation of an investigation which was commenced under a grant from the United States Interdepartmental Social Hygiene Board; the work has been under the general direction of Dr. Reid Hunt.

[2] Christiansen, THIS JOURNAL, (a) 43, 2202 (1921); (b) 44, 847 (c) 854 (d) 2334 (1922); (e) 45, 1316 (f) 1807 (1923).

[3] Ref. 2 a, p. 2206.

[4] Ref. 2 d, p. 2338.

conditions.[5] Hydrosulfite reduces the nitro group so rapidly that the reaction is very difficult to control. It seemed advisable, therefore, to study the action of a closely allied but less vigorous reducing agent on the nitro acid; sodium bisulfite was selected.[6]

Sodium bisulfite reacts with 3-nitro-4-hydroxyphenylarsonic acid in three distinctly different ways; the experimental conditions determine which of these processes shall occur. When the nitro acid or its sodium salt is refluxed in aqueous solution with bisulfite, the arsenical group is eliminated and o-nitrophenol is obtained.[7] When, however, a solution of the monosodium salt of the nitro acid and sodium bisulfite remains at room temperature, the main reaction product is 3-nitro-4-hydroxyphenylarsenious oxide. The mother liquor from this oxide gives a positive test for a sulfamic acid; therefore, although the arsonic acid group is the main point of attack under these conditions, the nitro group also suffers reduction to some extent.[8]

When the di- or trisodium salt of the nitro acid is treated at room temperature in water solution with sodium bisulfite, a slow, slightly exothermic reaction takes place, and the arylarsenious oxide mentioned above is not obtained. In this case, the reaction products are arsono-sulfamic acids which are exceedingly difficult to isolate on account of the ease with which they hydrolyze and their very great solubility.

It is possible, by means of a tedious, round-about process which involves the removal of practically all of the inorganic salts from the solution, to precipitate the organic material so that it is free from inorganic impurities. The organic material is a mixture of the barium and sodium salts of several arsonosulfamic and sulfo-arsonosulfamic acids.

During the study of this reaction, products containing an unsubstituted amino group could not be obtained from or detected in the reaction mixture; this is in accord with the results of Weil and his co-workers. As far as we know, this is the first recorded instance in which arsenicals containing sulfamic acid groups have been obtained.

From the mixture of salts referred to above, the barium salts of 2-hydroxy-5-arsonobenzenesulfamic acid (I), 2-hydroxy-3-sulfo-5-arsonobenzenesulfamic acid (II) and 2,2'-dihydroxy-5,5'-diarsonosulfone-anilide-N,N'-disulfonic acid (III) can be obtained by fractional precipitation from aqueous solution with alcohol. In one experiment a salt was isolated

[5] Ref. 2 c, p. 851.

[6] Weil and Moser [Ber., 55B, 732 (1922)] and Weil and Wassermann [Ber., 55B, 2533 (1922)] concluded that sulfamic and sulfo-sulfamic acids are the primary products of the bisulfite reduction of aromatic nitro compounds.

[7] King [J. Chem. Soc., 119, 1115 (1921)] obtained "excellent yields" of o-nitrophenol in this way.

[8] The formation of o-nitrophenol in boiling solution probably involves reduction of the arsonic acid to the nitrohydroxy-arsenious oxide prior to elimination of the arsenical group.

which appeared to be a derivative of 2,2'-dihydroxy-3-sulfo-5,5'-diarsono-sulfone-anilide (IV).

AsO_3H_2 AsO_3H_2 AsO_3H_2 AsO_3H_2

NHSO$_2$H   HO$_3$S   NHSO$_3$H

OH   OH

I   II   III

IV
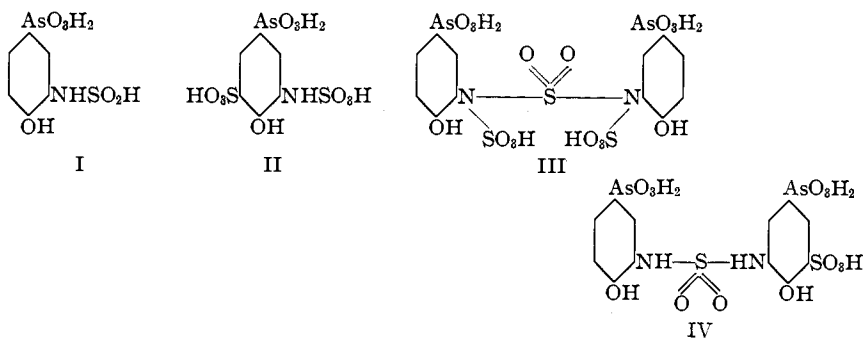
When hydrolyzed in acid solution, the salts of Acids I and III yield 3-amino-4-hydroxyphenylarsonic acid, and the salt of II yields 3-amino-4-hydroxy-5-sulfophenylarsonic acid; sufficient material was not available to permit the study of the hydrolysis products of the salt of IV. These salts are quite stable in the presence of neutral or alkaline reagents, but when acids are added hydrolysis of the sulfamic acid group occurs.

That the formation of sulfamic acids is not peculiar to 3-nitro-4-hydroxyphenylarsonic acid is evident from the fact that the analogous 4-methoxy acid is reduced under similar conditions to extremely soluble sulfamic acids. In this case, the reaction products were not isolated but qualitative tests definitely showed the presence of sulfamic acids.

The by-products that are formed during the hydrosulfite reduction of the nitro acid are present in a solution which contains a relatively large amount of 3-amino-4-hydroxyphenylarsonic acid.[9] Consequently, instead of studying the reduction products of these sulfamic acids, it is essential to determine the effect which they have on the reduction of 3-amino-4-hydroxyphenylarsonic acid to arsphenamine base.

When the pure amino acid, dissolved in sodium carbonate, is reduced with hydrosulfite, the arsphenamine base begins to separate while the system is being heated to the reduction temperature, and after an hour at 55–60°, the reaction is practically complete; also, the base precipitates in such a condition that it may be collected readily on a Büchner filter. The dried base dissolves readily in methyl-alcoholic hydrochloric acid, and the arsphenamine which is precipitated by the addition of ether contains, when dry, less than 0.5% of sulfur and is tolerated in doses of 150 mg. per kg. or higher.[10]

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[9] This amino acid can easily be obtained in 70% yields by hydrosulfite reduction of the nitro acid.

[10] In considering figures for the toxicity of arsphenamine it is necessary to keep in mind the fact that values found in different laboratories for the same specimen may vary greatly if different strains of rats and different diets are used. Thus, three samples of arsphenamine tested in this Laboratory and in Laboratory A gave the following values in mg./kg. for the tolerated dose.

This Laboratory	<100	120	140–150
Laboratory A	140	200	>200

Quite different results are obtained when mixtures of the salts[11] of the sulfamic acids and the amino acid are reduced. In these instances, the solutions remain clear for one quarter to one and a quarter hours after the temperature reaches 55°; after turbidity can be detected, the amount of insoluble matter increases slowly, and the reduction must be continued for three hours. Moreover, the insoluble products separate in such a finely-divided state that they can be collected by filtration only with difficulty. With the exception of the material obtained from the mixture of III and the amino acid, the dry arseno bases do not dissolve in methyl-alcoholic hydrochloric acid; the solids do, however, change in both color and texture. The arseno compounds which have been treated with alcoholic hydrochloric acid and dried are soluble in water, except in the case where a salt of I is used; the insoluble one dissolves in dil. sodium hydroxide solution. The products secured by the reduction of salts of I, II, III and IV in conjunction with the amino acid are tolerated in doses of 120, 70–80, 90 and 80 mg. per kg. and contain 5.53, 3.12, 1.47 and 3.84% of sulfur, respectively.

Evidently, the sulfamic acids derived from 3-nitro-4-hydroxyphenylarsonic acid when present in the same solution as the pure amino acid affect the reduction of the latter very markedly and cause the formation of arseno compounds which are more toxic and contain more sulfur than arsphenamine.

When the nitro acid is reduced with sodium hydrosulfite, the main reaction product is the amino acid; the mother liquor from the latter substance contains two by-products that are very difficult to isolate. By employing the process developed for the isolation of the substances formed by the action of bisulfite on the nitro compound, a mixture of the barium salts of the two by-products is obtained which can be separated into two fractions that are respectively soluble and insoluble in water; the former is by far the larger fraction. The constitutions of these two salts have not been established, but analytical data and qualitative tests show that the soluble portion is a tribarium salt of an hydroxysulfo-arsonobenzene-sulfamic acid and that the insoluble portion is a barium salt of an amino-hydroxy-sulfophenylarsonic acid. The sulfamic acid is not identical with II, because it does not yield 3-amino-4-hydroxy-5-sulfophenylarsonic acid when hydrolyzed in acid solution.

When a mixture of equivalent quantities of this sulfo-sulfamic acid, in the form of its sodium salt, and 3-amino-4-hydroxyphenylarsonic acid is reduced with sodium hydrosulfite the system, instead of remaining clear for some time at 55–60° as in the cases of the sulfamic acids described above, commences to deposit an insoluble arseno compound when the temperature reaches 46°, and a satisfactory yield is obtained in one and a half hours. The hydrochloride of this arseno base contains 2.64% of sulfur and is tolerated in doses of 60 mg. per kg. when injected, in alkalinized solutions, intravenously into rats. In this connection it is interesting to note that of the four sulfamic acids isolated from the bisulfite reaction, that which yields the most toxic arseno compound when reduced in the presence of the amino acid is the sulfo-sulfamic acid II.

On account of the small quantity of the insoluble barium salt available, it was impossible to mix it with an equivalent quantity of the amino acid from reduction; half an equiv-

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[11] The barium salts are first converted into sodium salts by treatment with sodium sulfate.

alent of the barium salt was used.   The reduction product forms readily; the final arseno compound contains 3.84% of sulfur and is tolerated in doses of about 120 mg. per kg.

Therefore, when 3-nitro-4-hydroxyphenylarsonic acid is reduced to the amino acid with sodium hydrosulfite, two by-products are formed; one is an hydroxysulfo-arsonosulfamic acid, and the other is a sulfo-amino-hydroxy-arsonic acid.   When reduced with the pure amino acid, both of these by-products yield arseno compounds that are more toxic and contain more sulfur than arsphenamine prepared from the pure amino acid.

A detailed investigation of the constitutions of the two by-products obtained by the action of hydrosulfite on the nitro acid would be very profitable, but studies in this field have been discontinued, temporarily at least, in this Laboratory.

King[12] has also examined the hydrosulfite reduction of the nitro acid to the amino acid and isolated 3-amino-4-hydroxy-5-sulfophenylarsenious oxide as a by-product by leaving the mother liquor from the crude amino acid in the ice box for a week.   It seems quite probable that this oxide is a secondary by-product resulting from the gradual reduction of the sulfoarsonic acid.

In this study arsenic was determined in arsonic acids by Ewins' method, and in arseno compounds by Lehmann's method.   The toxicity of the arseno compounds was determined by intravenous injection of alkalinized solutions into albino rats.

Experimental Part

A.   Reduction of 3-Nitro-4-hydroxyphenylarsonic Acid with Sodium Bisulfite

Reduction of the Monosodium Salt.—After the addition of 6 g. of sodium bisulfite, a solution of 5 g. of the nitro acid in 50 cc. of 0.38 N sodium hydroxide solution is left at room temperature; 3-nitro-4-hydroxyphenylarsenious oxide separates gradually as a yellow precipitate.   At the end of three days, the product is collected on a filter, washed, and dried in a vacuum; yield, 2.5 g.

The oxide is slightly soluble in water, alcohol and hydrochloric acid but readily soluble in dil. sodium hydroxide solution.   It reduces iodine and is reduced in the cold by hypophosphorous acid to a pale yellow, insoluble substance (dihydroxy-dinitro-arsenobenzene).   The dry powder causes sneezing.

Anal.   Calcd. for $C_6H_4O_4NAs$: As, 32.75.   Found (Ewins' method): 32.87; (iodine titration), 32.71.

When a small portion of the mother liquor from the oxide is acidified with hydrochloric acid, boiled, cooled, treated with sodium nitrite and added to alkaline β-naphthol, a purple color results.   Therefore, a portion of the original nitro compound was reduced to a sulfamic acid; the β-naphthol test is negative unless the acidified solution is heated.

Reduction of the Trisodium Salt.[13]—A solution of 20 g. of the nitro acid in 300 cc. of water containing 9.12 g. of sodium hydroxide is added to a solution of 42 g. of sodium bisulfite in 300 cc. of water, and the resulting solution is allowed to stand at room temperature.   As the solution smells strongly of sulfur dioxide after 46 hours, 2.8 g. of sodium hydroxide is added; at the end of an additional 24 hours a solution of 8 g. of

[12] Ref. 7, pp. 1107, 1415.

[13] A series of small-scale experiments showed that the optimum results are obtained with the reactants in the proportions given here.

bisulfite in 30 cc. of water is added.   The progress of this slow reaction may be followed by three different methods.   A test portion of the initial reaction mixture deposits a heavy precipitate of the nitro acid when acidified with hydrochloric acid; the quantity of precipitate gradually decreases in successive tests until precipitation does not occur. When the concentration of the reaction mixture is that specified above, this test becomes negative after only about 50% of the nitro compound has been reduced, because the nitro acid is appreciably soluble in water.   The tinctorial power of the nitro compound in the presence of alkali provides a very convenient means of following the reaction.   At the outset the reddish-orange reaction mixture colors filter paper deep yellow, but as the nitro group is reduced, the yellow color obtained on the paper becomes paler and paler until the tinctorial power of the solution is nil.   The solution, however, does not become colorless; it is light brown at the end-point.   Instead of following the disappearance of the nitro compound, the rate at which the quantity of sulfamic acids increases may be determined.   After the reaction has progressed somewhat, when a test portion is acidified with hydrochloric acid, boiled for about one minute and cooled, the addition of sodium nitrite and alkaline β-naphthol produces a red-violet color.   As the reaction proceeds, the depth of this color increases; at the end-point, successive tests are identical. Although the acidification test becomes negative after 24 hours, six days is required for complete reaction.

The clear, brown solution is treated with a hot solution of 150 g. of barium hydroxide octahydrate in 320 cc. of water, and the alkaline, yellow filtrate from the barium sulfite and sulfate is treated with a saturated aqueous solution of sodium carbonate to remove the barium.   The yellow filtrate from the barium carbonate contains the sodium salts of the organic acids, sodium carbonate and hydroxide.   It is concentrated first in a vacuum and then in a current of warm air (the solution remains at 35-45°) to 100 cc., and the cooled, pasty mass is washed repeatedly with 80cc. portions of alcohol. The residual oil contains the organic sodium salts and sodium carbonate; this oil can be be worked up in any of several ways.

1.   An aqueous solution of the oil is neutralized with acetic acid; the addition of alcohol precipitates the sodium salts of the organic acids as an oil which, after decantation of the supernatant liquid, is converted into a pale yellow powder by treatment with absolute methyl alcohol.   The yield is only 7.2 g., and the material is a mixture of the salts of several sulfamic acids.

2.   The oil is treated, in aqueous solution, with an excess of barium bromide; the filtrate from the barium carbonate contains sodium and barium salts of the organic acids and sodium and barium bromides.   From this solution the barium salts of the organic acids may be fractionally precipitated by the addition of successive portions of alcohol containing barium bromide.   In each case, the barium content is slightly low.

The first fraction is the di-barium salt of 2-hydroxy-5-arsonobenzenesulfamic acid (V).   It separates from the reaction mixture as a yellow, flocculent precipitate; the
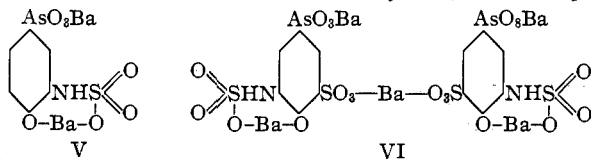
dry, yellow powder (3 g.) dissolves readily in water.

Anal.   Calcd. for $C_6H_4O_7NSAsBa_2$: S, 5.48; As, 12.8; Ba, 47.1. Loss at 105°: 14.6%. Found in dry material: S, 5.42, 5.33; As, 12.2; Ba, 44.2, 44.3.

When hydrochloric acid is added to a yellow, aqueous solution of V, the color disappears, and the clear solution gradually yields a precipitate of barium sulfate; precipi-

tation occurs rapidly when heat is applied. The cooled filtrate from the barium sulfate gives a bright red color when treated with sodium nitrite and alkaline β-naphthol; a cold, clear, acid solution of the barium salt becomes only faintly colored when tested with nitrite and β-naphthol. When a solution of 2 g. of this salt in 10 cc. of water is treated with 2 cc. of 20% sulfuric acid and boiled for 30 minutes, the filtrate from the barium sulfate yields slightly impure 3-amino-4-hydroxyphenylarsonic acid when evaporated to 10 cc., cooled, decolorized with vegetable carbon and treated with 20% sodium acetate solution until it is no longer acid to Congo Red; yield, 0.42 g., or 62%. Qualitatively, the hydrolysis product is identical with specimens of the amino acid prepared by the usual methods; it does, however, give a faintly positive test for sulfur, and the arsenic content is slightly low, 31.14% instead of 32.2%.

The second fraction is the tribarium salt of 2-hydroxy-3-sulfo-5-arsonobenzene-sulfamic acid (VI). It separates as an olive-green oil which, after removal of the aqueous alcohol, is converted into a white solid by treatment with a small quantity of water; by recrystallization from boiling water, this salt is obtained in white needles (0.8 g.).

Anal. Calcd. for $C_{12}H_6O_{20}N_2S_4As_2Ba_5$: S (total), 8.74; S (hydrolyzable[14]), 4.37; As, 10.2; Ba, 46.8. Loss at 105°: 21.3%. Found in dry material: S (total), 8.47; S (hydrolyzable) 4.41; As, 10.00; Ba, 45.4, 45.6.

This substance is difficultly soluble in cold water but readily soluble in cold, dil. hydrochloric acid; the cold, acid solution slowy deposits a precipitate of barium sulfate; when the solution is heated, precipitation occurs immediately. When the heated acid solution is cooled, filtered and treated with sodium nitrite, a yellow color develops which becomes deep red when alkaline β-naphthol is added; the clear, cold acid solution yields only a faint, brownish yellow coloration.

Hydrolysis of VI.—A suspension of 2 g. of the solid in 10 cc. of water is treated with 0.26 cc. of concd. sulfuric acid and the mixture heated on a water-bath for 45 minutes. The barium sulfate is removed by centrifuging and washed with 2 cc. of water. The reddish-brown solution is evaporated to about 1 cc. and cooled; coarse crystals of 3-amino-4-hydroxy-5-sulfophenylarsonic acid separate; yield, 0.4 g.

Anal. Calcd. for $C_6H_8O_7NSAs$: S, 10.2; As, 23.9. Found: S, 9.7; As, 23.0.
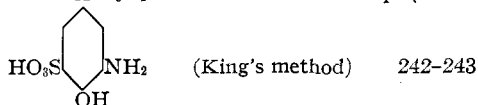
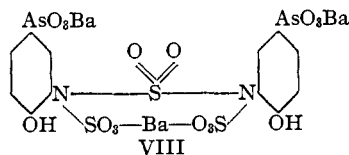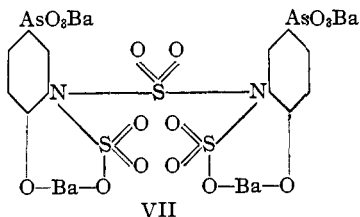|	M. p. (uncorr.) (decomp.)
(King's method)	242–243
Hydrolysis product of VI	243–244
Mixture	242.5–243.5

The third fraction appears to be the tetrabarium salt of 2,2'-dihydroxy-5,5'-di-arsonosulfone-anilide-N,N'-disulfonic acid (VII). It separates as a reddish oil which

VII   VIII

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[14] The hydrolyzable sulfur is determined by adding barium chloride to a dil. hydrochloric acid solution of the substance, digesting on a steam-bath and estimating the barium sulfate in the usual way.

is converted into a dense, yellow powder by treatment with absolute methyl alcohol; the dried product (12.6 g.) is readily soluble in water.

Anal. Loss at 105°: 10.2%. Calcd. for $C_{12}H_6O_{16}N_2S_3As_2Ba_4$: S, 7.79; As, 12.2; Ba, 44.6. Found in dry material: S, 7.11, 7.12; As, 11.2; Ba, 42.0.

When acidified with hydrochloric acid, the yellow aqueous solution of this salt becomes colorless but remains clear; the clear solution deposits barium sulfate gradually at room temperature but immediately when heated. When tested by the nitrite- β-naphthol method, the clear, cold, acid solution gives only a slight yellow color, whereas the boiled and cooled solution gives a deep red color. When hydrolyzed by the method used for V, this substance yields 3-amino-4-hydroxyphenylarsonic acid; the hydrolysis product contains 31.9% of arsenic (calcd., 32.2).

When carbon dioxide is passed into an aqueous solution of the tetrabarium salt (VII), precipitation of barium carbonate occurs, and the addition of alcohol to the filtrate from the latter precipitates the yellow tribarium salt (VIII).

Anal. Loss at 105°: 12.6%. Calcd. for $C_{12}H_8O_{16}N_2S_3As_2Ba_3$: S, 8.77; As, 13.7; Ba, 37.7. Found in dry material: S, 8.32; As, 13.3; Ba, 36.3.

3. When, instead of fractionally precipitating Salts V, VI and VII according to Method 2, a large quantity of alcohol is added at once to the aqueous solution containing the salts of the sulfamic acids and sodium and barium bromides, an oil is obtained which is a mixture of the barium salts mentioned above and a small quantity of analogous sodium salts. Treatment of this oil with absolute methyl alcohol converts it into a yellow solid which is a mixture of the barium salts V, VI and VII. The addition of ether to the methyl alcohol used in the solidification process precipitates an oil which, when reprecipitated from water solution with alcohol and treated with a *small* quantity of methyl alcohol, solidifies; too much methyl alcohol redissolves the material. As the ratio, arsenic: total sulfur: hydrolyzable sulfur, found for this material is 1:1.01:0.83, it is a crude sodium salt of an arsonosulfamic acid; 1 g. is obtained from 10 g. of the nitro acid; the test for barium is negative.

4. When the oil which is precipitated from the aqueous solution of the salts of the sulfamic acids and the bromides, and which is a mixture of the sodium and barium salts of the sulfamic acids (compare Method 3), is redissolved in water a solution is obtained which is analogous to that fractionated in Method 2 except that it is free from bromides. In one experiment, in which 150 g. of barium hydroxide was used instead of 160 g. to precipitate the inorganic sulfites and sulfates after reduction of the nitro compound, a solution free from bromides and all other inorganic salts was prepared in this way and fractionated. In this case the following yields were obtained from 20 g. of nitro acid: barium salts, 6.7 g. of II, 12.3 g. of III; sodium salts, 3.5 g. of I, 4.3 g. of IV (?); total, 26.8 g.

Reduction of 3-Nitro-4-methoxyphenylarsonic Acid.—A solution of 0.7 g. of 3-nitro-4-methoxyphenylarsonic acid in 6 cc. of water containing 0.4 g. of sodium hydroxide is treated with a solution of 1.04 g. of sodium bisulfite in 6 cc. of water, and the resulting yellow solution is allowed to stand at room temperature. A small test portion taken at the outset of the experiment yields a copious precipitate when acidified with hydrochloric acid; a similar test made after ten hours yields no precipitate; the yellow color has changed to brownish-orange. After 30 hours a test portion is cooled and acidified with hydrochloric acid; when sodium nitrite is added a slight yellow color results which changes to light orange upon addition of alkaline β-naphthol. When, however, an acidified sample is boiled, cooled and the nitrite is added a deep yellow color appears and this becomes very deep red after the β-naphthol has been added.

B.   Isolation of By-products Formed during the Hydrosulfite Reduction of 3-Nitro-4-hydroxyphenylarsonic Acid

A solution of 70 g. of 3-nitro-4-hydroxyphenylarsonic acid in 400 cc. of water containing 20 g. of sodium hydroxide is cooled, during vigorous stirring, to —3° in an efficient cooling mixture.   The addition of 175 g. of commercial sodium hydrosulfite causes the temperature to rise gradually to 10° and then increases rapidly to 46°; the color disappears during this vigorous reaction.   After 30 minutes the temperature has fallen to 5°, and a considerable quantity of 3-amino-4-hydroxyphenylarsonic acid has separated as a finely-divided, white solid.   A solution of 63 g. of hydrated oxalic acid[15] in 350 cc. of water is added, and the mixture is kept in the cooling-bath for an additional one and a half hours.   The crude amino acid is collected on a filter, washed several times with cold water and thoroughly extracted with water twice at room temperature.   After the crude amino acid has been dried in the air, it is obtained as a white powder weighing 40 g. (65%).

The mother liquor and first washings are combined and poured into a warm solution of 575 g. of barium hydroxide octahydrate in 1.2 liters of water; a heavy, white precipitate forms immediately.   After the mixture has stood in the ice box overnight, vegetable carbon is added, and the insoluble material is removed by filtration through a mat of carbon.   The red-brown, alkaline filtrate (about 3 liters) gives a slightly positive test for barium; it contains sodium salts of the organic acids, sodium hydroxide and a small quantity of barium hydroxide.   The solution is concentrated at a pressure of about 4 cm. of mercury to about 300 cc.; a slow stream of carbon dioxide is introduced during this process.   The liquor, which now contains some barium carbonate, is clarified and poured into 1.2 liters of alcohol; a red oil consisting of the sodium salts of the organic acids and sodium carbonate separates at once.   After the mixture has stood in the ice box overnight, the supernatant alcohol is removed by decantation; the residual oil is washed twice with alcohol, redissolved in 100 cc. of water, reprecipitated with 100 cc. of alcohol and washed with two 100cc. portions of alcohol.

A solution of the oil in 100 cc. of water is treated with a solution of 50 g. of barium bromide in 50 cc. of water; a heavy precipitate of barium carbonate forms.   As the filtrate from a small test portion still gives a slight precipitate when treated with barium bromide, a concentrated solution of 10 g. of the latter is added.   The barium carbonate is removed by centrifuging and washed twice with water.   An additional 10 g. of the bromide dissolved in 13 cc. of water is added to the clear, reddish-brown mother liquor in order that a large excess of this substance may be present, and 825 cc. of alcohol is added; a white, curdy precipitate forms.   After the material has stood in the ice box for four hours, the insoluble matter is collected on a filter, washed with two 100cc. portions of alcohol and air-dried.   The white product (10 g.) is free from sulfates and bromides and consists of the barium salts of the organic acids; the sodium and barium bromides remain in the alcohol.

This powder is extracted four times at room temperature with 30–35cc. portions of water; each extract is clarified by centrifuging and poured immediately into alcohol. A total of 400 cc. of alcohol is employed and all the extracts are added, as secured, to this single quantity of alcohol.   The white precipitate which forms is removed by filtration, washed with alcohol and dried in a vacuum.

The product (6.2 g.) is a barium salt of a sulfo-arsonosulfamic acid.   It is quite readily soluble in water and very soluble in cold, dil. hydrochloric acid.   The cold acid solution gradually deposits barium sulfate; this occurs instantly when heat is applied.

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[15] Oxalic acid is used because it yields an insoluble barium salt and because mineral acids must be avoided.

Analytical data indicate that this substance has the same empirical formula as VI, the tribarium salt of 2-hydroxy-3-sulfo-5-arsonobenzenesulfamic acid.

Anal. Loss at 105°: 11.2%. Calcd. for $C_{12}H_6O_{20}N_2S_4As_2Ba_3$: S (total), 8.74; S (hydrolyzable), 4.37; As, 10.2; Ba, 46.8. Found in dry material: S (total), 8.92; S (hydrolyzable), 4.91; As, 10.2; Ba, 44.0.

This material is more soluble and hydrolyzes more readily than VI, and it does not yield 3-amino-4-hydroxy-5-sulfophenylarsonic acid when hydrolyzed in acid solution.

An additional 0.6 g. of the soluble fraction is secured by re-extracting the residue (2.5 g.) from the first series of extractions. This time three 30cc. portions of water are used; each extract, after clarification, is poured at once into alcohol; 300 cc. of alcohol is taken, and the successive extracts are poured into this single portion of alcohol.

The grayish-white, insoluble fraction (1.7 g.) is readily soluble in cold, dil. hydrochloric acid; the very slight effervescence which occurs is probably due to the presence of a trace of barium carbonate. The acid solution remains clear for a short time and then becomes turbid; this occurs more rapidly when the solution is warmed, but the quantity of barium sulfate is comparatively slight in any case. Also, the analytical data show that only a small amount of hydrolyzable sulfur is present. This fraction is an impure salt of a sulfo-arsonic acid; the poor yield may be due to losses during the removal of the carbonate by the addition of barium bromide.

C. Effect of the Presence of Sulfamic Acids and the Above-Mentioned By-products on the Hydrosulfite Reduction of 3-Amino-4-hydroxy-phenylarsonic Acid

In studying the hydrosulfite reduction of the amino acid and mixtures of the pure amino acid with the various sulfamic acids, the general procedure has been the same in each case. Consequently, the procedure will be outlined and the results tabulated.

The sodium hydrosulfite is added to an aqueous solution of magnesium chloride which is being stirred mechanically at room temperature. About a minute later, the solution containing the arsenical or mixture of arsenicals to be reduced is added, followed by 1 g. of vegetable carbon. The clear solution obtained by filtration is stirred and heated during 15 minutes, to 55° and maintained at 55–60°. The reduction product is collected by centrifuging, washed twice with water and dried in a vacuum. The dry arseno base is suspended in absolute methyl alcohol and treated with methyl alcoholic hydrochloric acid (2.2 molecular equivalents). In those cases where the base dissolves, the alcoholic solution is filtered and added to 10 volumes of cold ether; otherwise the suspension is allowed to stand for 15 minutes and then added to the ether.

When the sodium salts of the sulfamic acids are available they are added, in aqueous solution, to a solution of the pure amino acid in dil. sodium carbonate solution. When the barium salts are to be employed, aqueous sodium sulfate is added to the aqueous solution or suspension of the barium salt 15 minutes before it is added to the amino acid solution. The barium sulfate is removed from the reduction mixture with the carbon.

In Tables I and II, the solution of sodium carbonate is saturated, the sodium sulfate solution contains 0.18 g. of the anhydrous salt per cc., Time I is the interval after 55° is reached before the reduction mixture becomes turbid and Time II is the total reduction time at 55–60°.

When arsphenamine is reprecipitated from aqueous solution, either acid or alkaline, with hydrochloric acid (1:1), any sulfur which, in the original

TABLE I

REDUCTION OF THE AMINO ACID AND MIXTURES OF IT AND THE SULFAMIC ACIDS

No.	Reaction mixture	Time I Hr.	II Hr.	Arseno base G.	Product Yield, g. color	As, %	S, %	Tol. dose Mg./kg.	S, % after HCl
I	1.8 g. of pure amino acid, 40 cc. of H₂O, 5 cc. of Na₂CO₃ soln.; 30 g. of Na₂S₂O₄, 130 cc. of H₂O, 5.3 g. of MgCl₂.6H₂O	0[a]	1.5	1.1[b]	1.1; pale yellow	30.2	0.14	150	0.06
II	Like I, but used crude amino acid	0[a]	1.5	1.05[b]	1.1; pale yellow	30.8	0.04	120	..
III	1.05 g. of Na salt of I, 10 cc. of H₂O; 0.5 g. of amino acid, 10 cc. of H₂O, 1.37 cc. of Na₂CO₃ soln.; 19 g. of Na₂S₂O₄, 82 cc. of H₂O, 3.4 g. of MgCl₂.6H₂O	0.25	3	0.39;[e] yellow	0.33; bright[d] yellow	26.1	5.53	120	1.52
IV	2.35 g. of Ba salt of II, 20 cc. of H₂O, 5.3 cc. of Na₂SO₄ soln.; 0.79 g. of amino acid, 15 cc. of H₂O, 2.2 cc. of Na₂CO₃ soln.; 30 g. of Na₂S₂O₄, 110 cc. of H₂O, 5.3 g. of MgCl₂.6H₂O	1.25	3	0.54;[e] green-yellow	0.52; bright yellow	30.0	3.12	70–80	1.81
V	2.4 g. of Ba salt of III, 15 cc. of H₂O, 5 cc. of Na₂SO₄; 0.9 amino acid, 20 cc. of H₂O, 2.5 cc. of Na₂CO₃ soln.; 30 g. of Na₂S₂O₄, 130 cc. of H₂O, 5.3 g. of MgCl₂.6H₂O	0.75	3	0.63;[b] bright yellow	0.53; deep yellow	29.4	1.47	90	0.08
VI	1.5 g. of Na salt of IV (?), 20 cc. of H₂O; 0.9 g. of amino acid, 20 cc. of H₂O, 2.5 cc. of Na₂CO₃ soln.; 30 g. of Na₂S₂O₄, 130 cc. of H₂O, 5.3 g. of MgCl₂.6H₂O	0.5	3	0.88;[e] green-yellow	0.84; bright yellow	28.3	3.84	80	0.43

[a] The arseno base appears before the temperature reaches 55°.

[b] The base dissolves in alcoholic hydrochloric acid.

[c] The base does not dissolve in alcoholic hydrochloric acid.

[d] Insoluble in water.

[e] Material is now soluble in water.

TABLE II

REDUCTION OF MIXTURES OF THE AMINO ACID AND THE BY-PRODUCTS, COMPARE B

I	2.82 g. of sol. fraction, 15 cc. of H₂O, 8 cc. of Na₂SO₄ soln.; 0.9 g. of amino acid, 20 cc. of H₂O, 2.5 cc. of Na₂CO₃ soln.; 30 g. of Na₂S₂O₄, 130 cc. of H₂O, 5.3 g. of MgCl₂.6H₂O	0[a]	1.5	0.86[b] deep yellow	0.85 pale yellow	30.3	2.64	60	0.65[c]
II	1.01 g. of insol. fraction, 6 cc. of H₂O, 3 cc. of Na₂SO₄ soln.; 0.45 g. of amino acid, 10 cc. of H₂O, 1.25 cc. of Na₂CO₃ soln.; 24 g. of Na₂S₂O₄, 104 cc. of H₂O, 4.2 g. of MgCl₂.6H₂O	0[a]	1.5	0.46[b] deep yellow	0.52 pale yellow	28.7	3.84	120	3.81

[a] Ref. a, Table I.

[b] Ref. c, Table I.

[c] The material is now tolerated in doses of 80 mg. per kg.

material, was present as sulfate or sulfamic acid is removed, whereas sulfur present in a sulfonic acid group attached to carbon is not removed. Thus, the sulfate of arsphenamine base (6.5% of sulfur) is converted into the dihydrochloride (0.86% of sulfur) by treatment with hydrochloric acid;[16] a slightly impure 3,3'-diamino-4,4'-dihydroxy-5-sulfo-arsenobenzene hydrochloride (5.12% of sulfur) contains 5.14% of sulfur after reprecipitation from alkaline solution with hydrochloric acid. The sulfur content of the reduction product after reprecipitation with hydrochloric acid is given in the last columns in Tables I and II.

Preparation of Barium Bromide.—During this work it was necessary to use an alcohol-soluble barium salt that contains an anion which also yields an alcohol-soluble sodium salt. Barium bromide satisfies these requirements and has been found useful in other cases where the chloride would be of little value. The preparation of this substance is a very simple matter.

One kg. of barium hydroxide octahydrate is mixed in an evaporating dish with 700 g. of ammonium bromide; the mixture becomes a thin paste and the temperature falls to —26°. The material is heated until ammonia is no longer evolved; water is added occasionally, when necessary, in order to keep the material pasty.[17] The mixture is evaporated until a thick mush is obtained which is extracted by boiling with four 1-liter portions of absolute methyl alcohol; only a small quantity of insoluble material remains. The extracts are evaporated to about 750 cc. and the barium bromide that separates is removed by filtration and washed with ether. The alcoholic mother liquor is added to 2 liters of ether; the barium bromide that precipitates is collected and washed with ether; total yield, 1,023 g.

Summary

1. Sodium bisulfite reacts with 3-nitro-4-hydroxyphenylarsonic acid in three ways. In boiling aqueous solution, the carbon-arsenic bond is severed and o-nitrophenol is obtained. At room temperature in aqueous solution, the monosodium salt of the nitro acid is reduced mainly to 3-nitro-4-hydroxyphenylarsenious oxide which separates as a yellow powder. The trisodium salt, however, is reduced under these conditions to salts of sulfamic acids which are exceedingly soluble in water and which can be isolated by a tedious process.

2. 3-Nitro-4-methoxyphenylarsonic acid is also reduced by sodium bisulfite to very soluble compounds of the sulfamic acid series.

3. This is the first recorded instance of the formation of salts of sulfamic acids containing arsenical groups.

[16] Ref. 2 e, p. 1319.

[17] When the mixture is allowed to become dry, the subsequent extraction with alcohol becomes quite difficult.

4. When mixtures of the sulfamic acids, which are formed by the action of sodium bisulfite on 3-nitro-4-hydroxyphenylarsonic acid, and 3-amino-4-hydroxyphenylarsonic acid are reduced with sodium hydrosulfite, the rate of formation of insoluble reduction products is much less than when the amino acid is reduced to arsphenamine base in the usual way, and the products separate from the solution in a different condition from that of arsphenamine base. The behavior of these bases in methyl alcoholic hydrochloric acid is also quite different from that of arsphenamine base. These products are more toxic and contain more sulfur than arsphenamine prepared from the amino acid.

5. When 3-nitro-4-hydroxyphenylarsonic acid is reduced with sodium hydrosulfite to 3-amino-4-hydroxyphenylarsonic acid, two by-products are formed which may be isolated and separated by means of their barium salts. Reduction of a mixture of one of these by-products and the pure amino acid yields a product which is much more toxic and contains more sulfur than arsphenamine prepared from the amino acid.

6. Barium bromide is recommended for the preparation of barium salts when alcoholic solvents are employed. The preparation of this bromide is described in detail.

BOSTON 17, MASSACHUSETTS

THE PREPARATION OF RAFFINOSE FROM COTTONSEED MEAL

BY D. T. ENGLIS, R. T. DECKER AND A. B. ADAMS

The occurrence and methods for the preparation of raffinose have been recently reviewed[1] and need no extended comment here. It has been shown by Hudson and Harding[2] that cottonseed meal is the cheapest and most convenient source, and the method of preparation which they worked out has made this rare sugar accessible, their yield of pure raffinose being about 2.5%. While their method gives good results with an experienced operator, it has been our experience that the rapid manipulations in the aqueous extraction of the meal and the intermediate formation and decomposition of the barium raffinosate, although a step of safety tending to make the method dependable, can cause difficulty. Recognizing a need for improvement, Harding[1] has suggested a new procedure consisting in extraction of the sugar with dil. aluminum sulfate, concentration to a small volume, precipitation of the aluminum sulfate with alcohol, clarification with basic lead acetate and finally separation of the raffi-

[1] Harding, Sugar, 25, 308 (1923).

[2] Hudson and Harding, THIS JOURNAL, 36, 2110 (1914),