[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, HARVARD MEDICAL SCHOOL]

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

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In Part III[2] of this series, purified and commercial varieties of sodium hydrosulfite were compared in respect to their use in reducing 3-nitro-4-hydroxy-phenylarsonic acid to relatively toxic arsphenamine and it was shown that the development of toxicity and the formation of a sulfonic acid derivative of arsphenamine during the reduction of the nitro acid are not due to impurities in commercial hydrosulfite. Extension of this comparison to the reduction of the nitro acid under the most favorable conditions and of the amino acid shows that the rate of reduction, the yield of arsphenamine base and the sulfur content are always greater when pure hydrosulfite is used instead of the commercial material.

The increased rate of reduction and yield are not due to the presence of additional hydrosulfite incident to the use of material which is 99% pure instead of 85%, because the use of commercial hydrosulfite in amounts 25% greater than those ordinarily used causes no increase in the rate of reduction or yield. Moreover, since the amount of commercial hydrosulfite ordinarily used constitutes a considerable excess, it would hardly be expected that the relatively slight increase in this excess resulting from the use of purified material would produce directly such marked changes.

It is more probable that they are due to some alteration of the condition of the reduction system brought about by the increased purity of the hydrosulfite. When hydrosulfite is dissolved in water, the solution becomes acid, and the purer the hydrosulfite, the greater is the resulting acidity. Also, since alkali stabilizes sodium hydrosulfite, it is not inconceivable that the commercial material might contain alkali carbonate. Pure hydrosulfite dissolves in dil. aqueous acetic acid without effervescence, whereas with some brands of commercial material vigorous effervescence occurs, while other brands give only slight effervescence. Addition of carbonate to pure hydrosulfite decreases the reducing power of the latter, thereby slowing down the rate of reduction and the yield of arsphenamine base. On the other hand, addition of a little acetic acid[3] to the system in which commercial hydrosulfite is to be used produces the opposite effect

[1] This is the eleventh of a series of studies on the properties contributing to the toxicity of arsphenamine being made under a grant from the United States Interdepartmental Social Hygiene Board to the Harvard Medical School; the work is under the general direction of Dr. Reid Hunt.

[2] Christiansen, THIS JOURNAL, 44, 2334 (1922).

[3] Inorganic acids cannot be used as they cause the decomposition of the hydrosulfite.

and the results are identical with those obtained with the purified material. The decreased reducing power due to the addition of carbonate and the increased power due to acetic acid is also evident when the nitro group is being reduced. The former slows down and the latter speeds up the reduction of the nitro group by hydrosulfite.

In Table I results are given comparing pure and commercial hydrosulfite and showing the effect of the addition of carbonate and acetic acid. The nitro acid was reduced under (a) the most favorable (b) the least favorable conditions[4] and the amino acid was dissolved in 2 molecular equivalents of caustic soda instead of carbonate so that it should be identical with the reduction of the nitro acid in regard to alkalinity. In recording the speed of reduction, it is designated as rapid or slow in comparison with the usual reduction using commercial hydrosulfite. The yields are given in weight of base per 2 g. of nitro acid or 1.8 g. of amino acid.

TABLE I

THE ACIDITY OF THE REDUCTION AND ITS BEARING ON THE ARSPHENAMINE

Source	Hydrosulfite	Speed of reduction	Yield G.	Sulfur content of base %	Sulfur content of dihydrochloride %
Nitro acid (a)	Pure	rapid	1.4	2.91	1.83
	Commercial	....	1.1	1.29	0.71
	Pure + Na_2CO_3	slow	0.9	..	0.82
	Com. + Na_2CO_3	very slow	0.56	..	..
	Com. + CH_3CO_2H	rapid	1.4	..	1.45
Nitro acid (b)	Pure	rapid	1.4	4.47	2.81
	Commercial	....	1.1	2.51	2.06
Amino acid	Pure	rapid	1.45	3.19	2.81
	Commercial	....	1.26	0.81	0.43
	Pure + Na_2CO_3	slow	1.25	..	0.12
	Com. + Na_2CO_3	very slow	0.8	..	..

The rate of reduction was determined in a series of experiments and the curves shown in Fig. 1 are typical. Ten-cc. samples of the rapidly stirred reaction mixture are removed with a pipet the end of which is not too small. The sample is cooled immediately in a freezing mixture to stop the reaction. After centrifuging, the supernatant liquor is decanted and the arsphenamine base is washed with 50% saturated salt solution and then with saturated salt solution. If water is used to wash the base, the latter shows a strong tendency to remain suspended and can be made to settle only by long centrifuging. The washed base is dissolved in 10 cc. of water and 2 drops of conc. hydrochloric acid. After the solution is transferred to an Erlenmeyer flask it is titrated with iodine solution using starch as an indicator. In expressing the results the volume of 0.09370 *N* iodine required is plotted against the time at which the sample

[4] THIS JOURNAL, **43**, 2207 (1921).

is taken. Time is counted from the moment of filtration at 30° and continued for 90 minutes after 55° is reached.

The curves of Fig. 1 show very distinctly the increased rate of reduction and yield obtained by use of pure hydrosulfite and the enormous effect produced by decreasing the acidity. It is possible to check these results very closely, so the method of sampling must be fairly satisfactory. The curves for the reduction of the nitro acid under the least favorable conditions and for the reduction of the amino acid are similar to those given in Fig. 1, that is, the curve for the pure hydrosulfite rises faster and higher than the one for the commercial hydrosulfite.

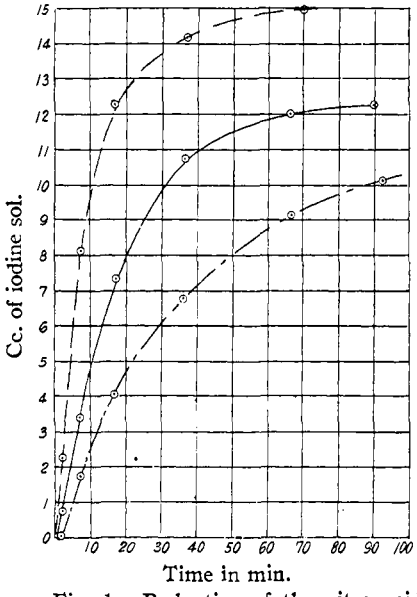

Fig. 1.—Reduction of the nitro acid under the best conditions to arsphenamine base.

——·——·——Purified $Na_2S_2O_4$

————Commercial $Na_2S_2O_4$

——·——·——Purified $Na_2S_2O_4 + Na_2CO_3$

From Table I it can readily be seen that when pure hydrosulfite is used for reduction, the sulfur content rises very sharply. In as much as the increase occurs irrespective of whether the nitro or the amino acid is reduced, the additional sulfur is introduced largely after the nitro group has been reduced, that is, during the reduction of the amino acid; this sulfur is present mainly as the sulfate of arsphenamine base and probably to some extent in the form of arsenic sulfur compounds.[5] Since the excess sulfur is combined as a salt of arsphenamine base, it should vary in the same direction as the acidity of the reduction. The experiments recorded in Table I show that when the acidity of the reductions with pure hydrosulfite is decreased by the addition of carbonate the excess of sulfur is not introduced and the products have practically the same sulfur content as specimens made by the usual reduction with commercial hydrosulfite, and when acetic acid is added to reductions made with commercial material, the sulfur content of the product rises. That the acidity is affecting the salt formation between the base and sulfur acids and that this is the main cause of the excessive sulfur are shown by the fact that if a reduction is made which would result in a high sulfur content due to the acidity of the mixture and if sodium carbonate is added after the reduction has reached completion, the sulfur content is as low as when the carbonate is added at

[5] THIS JOURNAL, **44**, 852 (1922).

the beginning of the reduction. Moreover, addition of sodium carbonate at this point causes no decrease in yield.

In consideration of the use to which arsphenamine is put, it is of prime importance to be sure that, even though changes in the reduction increase the yield, the toxicity of the product does not increase. Therefore, it is very gratifying to note that the use of a small quantity of acetic acid in conjunction with commercial hydrosulfite causes no increase in toxicity of the product. Such specimens are tolerated by albino rats in doses of 130–140 mg./kg. and contain 30.5% of arsenic. The fact that these samples, containing 1.45% of sulfur, are tolerated in such large doses brings out again the absence of any relation between the total sulfur content and the toxicity. The amount of acetic acid used must be controlled very carefully, and the optimum quantity has been found to be 1 cc. of glacial acid per g. of nitro acid to be used. The acetic acid is added to the cold dilute aqueous solution of the magnesium chloride just before the commercial hydrosulfite is added.[4] When less is used the maximum increase in yield is not obtained, and a larger amount (2 cc. per g.) causes a sudden increase in the toxicity of the product.

In discussing the distribution of sulfur in arsphenamine in relation to the mode of reduction, it was stated[5] that part of the sulfur in specimens made by reduction of the nitro acid with commercial hydrosulfite should be held in the same mode of combination as that present in samples made from the amino acid and that all samples made under those conditions should contain approximately 0.43% of sulfur in this state of combination. When the sulfate of arsphenamine base is suspended in water and treated gradually with hydrochloric acid it dissolves, and when this solution is poured into 1:1 hydrochloric acid the dihydrochloride is precipitated. Thus, 1.2 g. of the sulfate containing 29.6% of arsenic and 6.5% of sulfur (calc. for $C_{12}H_{12}O_2N_2As_2.2H_2O.H_2SO_4$: As, 30.0%; S, 6.4%) was dissolved in 30 cc. of water and 1 cc. of hydrochloric acid (d., 1.19). The clear solution was poured into 60 cc. of cold 1:1 hydrochloric acid. After the mixture was filtered, the precipitate washed with cold 1:1 hydrochloric acid and dried in a vacuum over sodium hydroxide, 1 g. of dihydrochloride containing only 0.86% sulfur was obtained.

TABLE II

REMOVAL OF SULFUR BY TREATMENT WITH HYDROCHLORIC ACID

Source of arsphenamine	S in dihydrochloride %	S present after treatment with HCl	Diff.
Amino acid	0.43	0.06	0.37
Nitro acid (a)	0.71	0.42	0.29
Nitro acid (b)	2.13	1.79	0.34

On the other hand, sulfur present as the sulfonic acid derivative of arsphenamine is not removed by treatment with hydrochloric acid. The

5-sulfonic acid derivative of arsphenamine base, prepared by reduction of a mixture of the amino arsonic acids with hypophosphorous acid, was treated repeatedly with 1:1 hydrochloric acid. After the product had been dried thoroughly it contained 7.47% of sulfur. In Table II values are given showing the amount of sulfur in various samples of arsphenamine before and after treatment with hydrochloric acid. All of the samples were prepared with commercial hydrosulfite without the use of acetic acid or sodium carbonate.

These results show clearly that the sulfur present in arsphenamine prepared from the amino acid is completely removable by treatment with hydrochloric acid and that similar treatment removes approximately the same amount of sulfur from specimens prepared from the nitro acid. The conclusions stated above are substantiated very well by these experiments.

The ease with which arsphenamine dissolves in water is extremely variable and depends partly upon the procedure used in converting the base into the dihydrochloride. Thus, when a methyl-alcoholic-hydrochloric acid solution of the base is precipitated by ether, the resulting hydrochloride is readily soluble in cold water, whereas the precipitation of a solution of the same base in dil. aqueous hydrochloric acid with conc. hydrochloric acid gives a product which is very difficultly soluble in cold water. The differences produced in this way have been discussed by Sherndal[6] who considers arsphenamine to be capable of existing in varying degrees of polymerization and that the difficultly soluble specimens precipitated from aqueous solution are highly polymerized while the readily soluble products are only slightly polymerized. The present research has shown that the ease of solution also depends upon the conditions under which the nitro or amino acid is reduced to arsphenamine base. When pure hydrosulfite is used the resulting arsphenamine is much more readily soluble and the solution is less viscous than when commercial hydrosulfite is used. This is due to increased acidity resulting from the increased purity of the hydrosulfite, because if a little acetic acid is used in conjunction with the commercial material the ease of solution of the arsphenamine is increased.

Summary

The use of pure instead of commercial hydrosulfite for the reduction of 3-nitro-4-hydroxy-phenylarsonic acid or the corresponding amino acid to arsphenamine base results in a greater rate of reduction, a larger yield, and a higher sulfur content. These factors are controlled by the acidity of the reduction mixture, and the addition of sodium carbonate to pure hydrosulfite slows down the rate of reduction, decreases the yield, and lowers the sulfur content. The use of acetic acid with commercial hydrosulfite has the opposite effect.

[6] Sherndal, *J. Lab. Clin. Med.*, **7**, 723 (1922).

Conclusions previously drawn regarding the distribution of the sulfur in arsphenamine have been substantiated.

The ease of solution of arsphenamine in water depends partly upon the acidity of the reduction medium during the formation of arsphenamine base. Specimens prepared by use of pure hydrosulfite are much more readily soluble than similar ones in the preparation of which commercial hydrosulfite has been used.

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PREPARATION OF THE MERCURY COMPOUNDS OF THE PHENYL HALIDES

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The preparation of the simple halogen-benzene-mercury compounds is not recorded in the literature. The problem can be attacked in at least two ways: (1) by the direct mercuration of the phenyl halide, such as the treatment of the phenyl halide with mercuric acetate, resulting in the replacement of a hydrogen atom of the benzene ring by mercury; (2) by the indirect introduction of mercury, that is, the replacement by mercury of some group already present in the benzene ring, such as the sulfinic acid group.

In 1920 Kharasch[2] found that when phenyl halides are heated with mercuric acetate, mercury compounds are formed. Since then the direct mercuration of the phenyl halides has been more closely investigated by the writer. It has been found that although the *p-halogen-phenylmercuric acetates* can easily be isolated in fairly good yield, the separation of the remaining *ortho* and *meta* compounds, and possibly other products, is extremely difficult, and requires a tedious process of fractional crystallization. The reaction is one of great theoretical interest, but as a source of mercury compounds of the phenyl halides it thus far appears to be impractical.

In the hope of finding a more expedient method for preparing these compounds the reaction of heating the mercuric salt of the halogen-ben-

[1] This work was done by Martin E. Hanke as National Research Fellow in Organic Chemistry at the University of Chicago from September, 1921, to August, 1922.

[2] Private communication.