except in cases where the amount of sulfonic acid was very high, could be filtered without the sulfonic acid separating. The material was precipitated by ether in the usual way.

Summary

1. A careful study of the mode of synthesis, toxicity and sulfur content shows that arsphenamine prepared (*a*) from the amino acid is the least toxic and has the lowest sulfur content; (*b*) from the nitro acid under the most favorable conditions is slightly more toxic and has a slightly higher sulfur content; (*c*) from the nitro acid under least favorable conditions is much more toxic and has a much higher sulfur content.

2. There is no direct relation between the total sulfur and the toxicity.

3. Only the sulfur in excess of that introduced when the nitro acid is reduced under the most favorable conditions has any great effect upon the toxicity.

4. Only from those preparations made from the nitro acid under the least favorable conditions can the sulfonic acid derivative of arsphenamine described by King be isolated, and the amount which the sulfur content can be lowered by removal of this substance is nearly the same as the excess sulfur introduced by improper reduction of the nitro group.

5. The presence of this sulfonic acid derivative cannot account for the whole of the high toxicity which is obtainable.

I wish to express my appreciation of Dr. Reid Hunt's work in determining the toxicity of the substances used in this paper, and of Mr. Arthur J. Norton's assistance in determining the correction to be applied in the sulfur analyses.

BOSTON, MASSACHUSETTS

[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. II[1]

BY WALTER G. CHRISTIANSEN

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While comparing high sulfur-containing, highly toxic arsphenamine obtained by the hydrosulfite reduction of 3-nitro-4-hydroxyphenylarsonic acid[2] with high sulfur-containing samples[3] prepared from known mixtures

[1] This is the seventh 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 who is also responsible for the biological tests reported in this paper.

[2] This type of arsphenamine will be referred to as arsphenamine I; for preparation, see THIS JOURNAL, **43**, 2202 (1921).

[3] Arsphenamine II; for preparation, see preceding paper.

of 3-amino-4-hydroxyphenylarsonic acid and its 5-sulfonic acid,[4] it was found that although both deposit the 5-sulfonic acid derivative of arsphenamine from a conc. methyl alcohol solution there are several points of difference between the two types of preparations. Some of these are the rate at which the sulfonic acid separates from the alcohol solution, the effect of temperature on the formation of the precipitate, the ease of separation of the precipitate from the mother liquor and the rate of death of rats when the substances are injected intravenously.

Fargher and Pyman[5] and later King,[4] working with English commercial samples, reported that the solid separates only slowly from alcoholic solution. This observation was found to be true, many solutions of high sulfur arsphenamine showing no signs of turbidity for a number of hours. When variations in the temperature were made marked changes were produced in the rate of precipitation. In the experiments on arsphenamine I recorded in the following table the alcoholic solutions were made at 0° and kept in completely filled stoppered tubes.

TABLE I

PRECIPITATION OF ARSPHENAMINE I FROM ALCOHOLIC SOLUTION

Ratio of Arsph. to CH_3OH G.: Cc.	S Content %	Temp. ° C.	Time in hours 1/2	1	2	4	20	45	Effect of warming to 45°
1 : 5	1.40	0	0	0	0	0	0	0	
1 : 4	1.56	0	0	0	0	0	0		
1 : 4	1.78	0	0	0	0	0	0	−	++ in 20 min.
1 : 4	1.87	0	0	0	0	0	+		
1 : 3	..	0	0	0	0	0	+	+++	
1 : 4	1.47	8	0	0	0	0	+		+++ in 15 min.
1 : 5	1.40	23	0	0	0	0	+++		
1 : 4	1.87	R.T.[a]	0	0	0	—	++		
1 : 3	1.64	R.T.	0	0	+	++	++		
1 : 3	..	R.T.	0	+	++	+++			
1 : 5	2.04	45	+	+++					
1 : 5	1.40	45	0	+	++				
1 : 3	..	45	+++						

+ Turbid.
++ Slight precipitate.
+++ Heavy precipitate.
[a] Room temperature.

These results show that at any one temperature precipitation is more rapid from a concentrated than from a dilute solution, and that at any one concentration precipitation becomes faster as the temperature is raised. Temperature has the greater effect as a 1:5 solution at 23° or 45° separated faster than a 1:3 solution at 0° or room temperature respectively. Also,

[4] King, *J. Chem. Soc.*, **120**, 1107 (1921).
[5] Fargher and Pyman, *ibid.*, **117**, 370 (1921).

the immediate precipitation caused by warming a solution which has only undergone a slight change at 0° shows the importance of the temperature.

A similar series of experiments (Table II) was run with arsphenamine II, *i. e.*, arsphenamine whose main sulfur impurity is necessarily the 5-sulfonic acid derivative in varying amounts.

TABLE II

PRECIPITATION OF ARSPHENAMINE II FROM ALCOHOLIC SOLUTION

Ratio of Arsph. to CH_3OH	S Content	Temp.	Time in hours					Warm to 45°
G.: Cc.	%	°C.	1/2	1	2	4	20	
1:4	2.10	0	*a*		+++			
1:6	1.75	0	+		+++			
1:4	1.50	0	0	+	+++			
1:4	1.23	0	0	0	0	0	+++	
1:4	1.03	0	0	0	0	+	+++	
1:4	0.99	0	0	0	+	+++		
1:4	0.95	0	0	0	0	0	0	+in 2 hrs.
1:4	1.23	R.T.	0	+	+++			
1:4	0.99	R.T.	+	+++				

[a] Precipitation set in before all the material dissolved.

A preparation of this type and containing 1.75% of sulfur if added to 4 parts of methyl alcohol at room temperature will not be completely dissolved before a precipitate starts to form and within 30 minutes a heavy precipitate will have formed. From the above table (II) it is evident that arsphenamine II, whose sulfur content equals that of arsphenamine I but whose sulfur has been introduced nearly wholly as sulfonic acid, precipitates very rapidly from conc. methyl alcohol solutions. This type of preparation even when containing a much lower sulfur content than the average toxic arsphenamine I, deposits a precipitate much more rapidly. Again, the precipitation is more rapid at room temperature than at 0°. However, it appears that in this series warming a solution which has given no precipitate in 20 hours at 0° does not cause the sudden formation of a heavy precipitate as was the case in Table I.

The addition of methyl alcoholic-hydrochloric acid to alcoholic arsphenamine solutions also increases the speed of precipitation of the sulfonic acid derivative (Table III).

Experiments were made to determine the extent to which the sulfur content of arsphenamine could be lowered by removal of the sulfonic acid by warming and by the addition of hydrochloric acid at 0° (Table IV). Apparently arsphenamine I upon standing for a long time at room temperature, a short time at 45°, or 20 hours with a small amount of acid

TABLE III

EFFECT OF ADDITION OF METHYL ALCOHOLIC HYDROCHLORIC ACID TO ARSPHENAMINE SOLUTIONS

Ratio of Arsph. to CH_3OH to HCl G. : Cc. : G.	S Content %	Temp. °C.	Time in hours 1/2	1	2	4	20	27
(Arsphenamine I)								
1 : 4 : 0	1.78	0	0	0	0	0	0	
1 : 4 : tr	1.56	0	0	0	0	0	+	++
1 : 4 : 0.05	1.78	0	0	0	0	0	+++	
1 : 4 : 0.05	1.87	0	0	0	0	—	+++	
1 : 3 : 0.04	1.64	R.T.	0	0	+++			
(Arsphenamine II)								
1 : 4 : 0.1	1.23	0	+	+++				
1 : 4 : tr.	1.03	0	0	0	0	++	+++[a]	

[a] After 10 hrs.

TABLE IV

REMOVAL OF SULFONIC ACID

Ratio of Arsph. to CH_3OH to HCl G. : Cc. : G	Expt.	Temp. °C	Time Hours	Sulfur content Original %	Sulfur content CH_3OH-soluble %
(Arsphenamine I)					
1 : 4 : 0	2	R.T.	45	1.70	1.27
1 : 4 : 0	3	0 and 45	20 5	1.71	1.29
1 : 4 : 0.05	1	0	20	1.87	1.22
(Arsphenamine II)					
1 : 4 : 0	4	0	20	1.48	0.91

lowers the sulfur content of the material remaining in the alcohol to the same value, 1.26%. However, the decrease never goes as far as in the case of arsphenamine II.

It was previously stated that the low sulfur-containing, slightly toxic arsphenamines prepared under the most favorable conditions from the nitro acid[6] gave no precipitate from alcoholic solution. Action of heat and hydrochloric acid has been investigated on these solutions. A 1:4 solution of such a sample (0.58% of sulfur) after 20 hours at 0° and 5 hours at 45° showed no trace of yellow precipitate; a similar solution containing 0.05 g. of hydrochloric acid after 23 hours at 0° contained not the slightest precipitate. In addition to proving that arsphenamine prepared in this way cannot be forced to deposit sulfonic acid from alcoholic solutions, these experiments prove that these accelerating processes cause only the deposition of that portion of the arsphenamine containing the sulfur from alcoholic solutions of high sulfur arsphenamine.

When centrifuging the alcoholic solutions in order to remove the precipitated sulfonic acid derivative, it has been found quite difficult to clarify

[6] See THIS JOURNAL, 43, 2202 (1921) for method of preparation.

the liquor when arsphenamine I is being examined, whereas solutions of arsphenamine II deposit the sulfonic acid in such a way that the liquor becomes clear very readily. When hydrochloric acid was used to accelerate the precipitation from Type I the clarification became much easier.

From biological experiments it appears that arsphenamine II causes very slow death while type I frequently kills with great rapidity and always in smaller doses.

All the phenomena reported here and the biological experiments reported in a previous paper show that arsphenamine I differs considerably from arsphenamine II, *i. e.*, arsphenamine which would have low toxicity and low sulfur if the sulfonic acid had not been mixed with the pure amino acid. The great effect produced on the rate of separation of the 5-sulfonic acid derivative from alcoholic solutions of arsphenamine I by the application of heat or small amounts of hydrochloric acid, and the rapid formation of heavy precipitates when solutions which have changed little at 0° are heated, and the fact that solutions of arsphenamine II which do not give a heavy precipitate at 0° will not do so at 45°, might indicate that this substance is not present as such but is produced by rearrangement of some unstable substance.

However, the 5-sulfonic acid derivative of arsphenamine may be present as such in type I and the slow separation which can be greatly accelerated by heat or acid may be due to the colloidal nature of the material. Inasmuch as alcoholic solutions of arsphenamine II, which must contain the sulfonic acid as such on account of the method of preparation, also deposit the sulfur compound more rapidly when warmed or when acidified, the second suggestion seems the more plausible. Very little is known concerning the colloidal nature or state of molecular aggregation of arsphenamine and considerable work must be done along these lines before the problem can be fully understood.

When alcoholic solutions of arsphenamine I are kept at 45° for 20 to 24 hours and are then poured, without separation of the precipitate, into ether, the material is completely recovered with little alteration in sulfur content but with a lower toxicity (Table V).

TABLE V
RECOVERY WITH ETHER

Ratio of Arsph. to CH_3OH	Temp.	Time	Tol. dose		Sulfur content	
			Original	Final	Original	Final
G.: Cc.	°C.	Hours	Mg./kg.		%	
1:5	45	24	50	*ca.* 80	2.04	
1:5	45	20	*ca.* 60	90	1.40	1.34

The substance recovered cannot be redissolved in methyl alcohol and acts like a mechanical mixture of arsphenamine and its 5-sulfonic acid. The toxicity has also become the same as that found for arsphenamine II[3]

the alcoholic solution of which deposits a precipitate rapidly at 0°. Heating, therefore, whether by causing a rearrangement or an alteration in colloidal properties, destroys some of the differences between types I and II.

To explain the existing phenomena it will be necessary to obtain a more definite understanding of the mechanism of the reaction and side reactions which take place when the nitro group is reduced and the processes which may occur when the by-products undergo the subsequent reactions. This work is well under way at the present time.

Summary

Certain chemical and biological differences between high sulfur, highly toxic arsphenamine obtained from the nitro acid and arsphenamine prepared by the reduction of mixtures of the amino acid and its 5-sulfonic acid have been pointed out. Two suggestions have been made regarding the cause of these differences and work has been started to study the mechanism by which the sulfur compounds are formed as a result of hydrosulfite reduction of the nitric acid.

I wish to thank Dr. Reid Hunt for determining the toxicity of the specimens employed in this work.

BOSTON, MASSACHUSETTS

[CONTRIBUTION FROM THE LABORATORY OF PLANT CHEMISTRY OF THE DEPARTMENT OF BOTANY, UNIVERSITY OF MICHIGAN]

THE CATALYTIC HYDROGENATION OF DEXTRO GLUCOSE. PRELIMINARY NOTICE

BY W. E. CAKE

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In view of all the recent work in the field of catalytic hydrogenation, it is remarkable that there are no accounts in the literature of the catalytic reduction of the sugars to the sugar-alcohols. It would be surmised that in such a reactive molecule as a straight chain hydroxy-aldehyde or hydroxy-ketone, the aldehyde or ketone group would readily lend itself to reduction by hydrogen in the presence of a catalyst.

The behavior of the sugars in neutral and acid solutions is such as to lead to the conclusion that they are not, for the most part, in a straight chain form, but that they exist in a γ-oxide ring form, in which the molecule does not contain an active aldehyde or ketone group. It is usually considered that the two forms are in equilibrium, and that the equilibrium point is far over to the γ-oxide ring side.

In alkaline solution, on the other hand, it seems as though there is quite an appreciable amount of the straight-chain forms present, either, in the case of the aldoses, as the aldehyde hydrate or, what is more likely, as the aldehyde itself in equilibrium with the aldehyde hydrate, together