A MODIFICATION OF SAKAGUCHI'S REACTION FOR THE QUANTITATIVE DETERMINATION OF ARGININE.

By C. J. WEBER.

(From the Department of Experimental Medicine, the University of Kansas, School of Medicine, Kansas City.)

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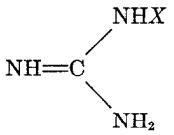
The extremely sensitive color reaction given by certain guanidine derivatives with α-naphthol and sodium hypochlorite, which was observed by Sakaguchi (1925), has been extensively employed as a qualitative test for arginine. The greatest obstacle in a quantitative application of this reaction is its slow rate of color development with the probable destruction of some of the guanidine group by the hypochlorite. When sodium hypobromite is employed instead of sodium hypochlorite, the color development with arginine is practically instantaneous, although colorimetric readings are not possible, since the color begins to fade immediately and almost disappears in 10 minutes. However, the addition of urea immediately after the color development has taken place stabilizes the color sufficiently to permit colorimetric comparisons. This modification of Sakaguchi's reaction is being studied because of its possible application to the colorimetric determination of arginine and certain other guanidine derivatives.

While our studies are not as complete as those made by Poller (1926) on Sakaguchi's reaction, we found that arginine, glycocyamine, and methylguanidine give an intense red color with this modified reaction; while creatine, creatinine, urea, glycocyamidine, guanidine, and *as*-dimethylguanidine[1] do not give any color reaction. No compounds have been found that give the characteristic

[1] One commercial preparation of *as*-dimethylguanidine sulfate gave color corresponding to a probable content of methylguanidine sulfate as an impurity of 4.7 per cent. By recrystallization from 70 per cent alcohol, a preparation of *as*-dimethylguanidine sulfate was obtained which gave no color.

red coloration given by the reacting guanidine derivatives. It is perhaps safe to predict that derivatives having the following type formula



where X is either a fatty acid or alkyl radical, will produce color with this reagent.

Arginine shows a color reaction in a dilution of 1:2,500,000 or 0.0004 mg. per cc. Molar equivalents of arginine and methylguanidine produce equal amounts of color. Glycocyamine, or guanidine acetic acid, produces only 70 per cent as much color as would be expected when molar equivalents of methylguanidine and glycocyamine are compared. Since glycocyamine is an amphoteric compound, a part probably exists as an "inner salt" which does not respond to Sakaguchi's reaction.

Protein gives this color reaction because of its arginine content, but no colorimetric determination of arginine in unhydrolyzed protein is possible, since the color values are much less than the values expected from the actual arginine content of the protein. The application of the method to hydrolyzed protein is being studied. The fact that protein responds to this reaction makes it imperative that protein be absent from biological solutions be ng tested for arginine or other guanidine derivatives.

The modified reaction has been tested from two standpoints: first, the amounts of the various reagents and the conditions necessary to give maximum color production and the greatest accuracy in colorimetric readings; second, the effect of certain substances that are usually present or added during the preparation of solutions of biological material on the color development given by arginine.

The solutions used in this determination are as follows: (1) sodium hydroxide, 10 per cent; (2) α-naphthol, 0.02 per cent, made by diluting 20 cc. of 0.1 per cent α-naphthol in 95 per cent alcohol to 100 cc. with distilled water; (3) sodium hypobromite, made by dissolving 2 gm. of bromine in 100 cc. of 5 per cent sodium hydroxide; (4) urea, 40 per cent.

The α-naphthol and hypobromite solutions are kept in amber bottles. The hypobromite solution will keep for about 2 weeks, while the α-naphthol solution will keep for at least 2 months.

The procedure is as follows: 5 cc. of the solution to be tested are placed in a test-tube kept in an ice bath. Add 1 cc. of sodium hydroxide and 1 cc. of α-naphthol and mix. The contents of the tube are cooled in the ice bath for 2 or 3 minutes, following which 0.1 to 0.2 cc. of sodium hypobromite is added, the tube shaken, and within 4 to 6 seconds, 1 cc. of urea is added and thoroughly mixed. The color is developed in the standard in the same manner and the colorimetric comparisons completed within 5 minutes.

Prior to performing the quantitative determination of arginine in an unknown solution, the amount of hypobromite necessary for complete color development must be determined. This is done by taking a series of tubes containing 5 cc. of the unknown and increasing the amount of hypobromite by 0.1 cc. portions. The standard should, however, contain only the amount of hypobromite necessary for complete color development, which is 0.1 cc. when a freshly prepared hypobromite solution is used.

The addition of various solutions is made more easily by using burettes. 10 cc. pipettes have been converted into pinch-cock burettes for this purpose.

The quantity of sodium hydroxide employed may be varied considerably if a sufficient amount is added to give the necessary alkalinity to the solution which is to be tested.

An excess of α-naphthol produces a yellow color, the intensity of which depends upon the amount used. However the quantity of α-naphthol necessary to give complete color development with the arginine concentrations employed, gives only the faintest trace of yellow and causes no disturbance in color matching, even when very dilute solutions are compared.

The greater the excess of hypobromite used in developing the color, the more quickly fading occurs, even if urea is added to destroy the excess hypobromite. When pure arginine solutions are tested, 0.1 cc. of hypobromite will give complete color development and, with the addition of an excess of urea, the color is stable for at least 10 minutes. With 0.2 cc. of hypobromite, readings must be made in 5 minutes to obtain comparable results. With

0.3 cc., the readings are 5 to 10 per cent too low and with 0.5 cc. hypobromite the color fading is too rapid for good comparisons when compared with a standard to which 0.1 cc. of hypobromite was added for color development.

The presence of substances like urea, creatinine, and reducing reagents which react with hypobromite, naturally requires the addition of more hypobromite for complete color development. However, the addition of an increased quantity of hypobromite in these cases does not appreciably influence the stability of the color produced as long as only the minimum quantity of hypobromite necessary for maximum color production is added.

TABLE I.

Determination of Arginine in Aqueous Solutions.

Arginine content of solutions.	Arginine found in individual determinations.				Maximum deviation from actual arginine content.	Deviation of average value from actual.
mg. per 1000 cc.	*mg. per 1000 cc.*	*mg. per 1000 cc.*	*mg. per 1000 cc.*	*mg. per 1000 cc.*	*per cent*	*per cent*
2.0	2.00	1.97	1.96	1.98	−2.00	−1.00
2.8	2.76	2.72	2.74		−2.86	−2.14
3.6	3.62	3.60	3.61		+0.55	+0.28
2.2	2.26	2.24	2.21	2.23	+2.73	+1.82
4.2	4.28	4.32	4.30	4.32	+2.86	+2.62
5.2	5.22	5.25	5.23	5.22	+0.96	+0.58
1.6	1.64	1.66	1.61	1.63	+3.75	+2.48
10.0	10.01	10.10	10.06	10.03	+1.00	+0.50

The addition of urea after the color development has taken place causes the color to become sufficiently stable to permit colorimetric readings. Without the addition of urea, the color produced with 0.02 mg. of arginine in 5 cc. and 0.3 cc. of hypobromite fades completely in 5 to 10 minutes, while with the addition of urea it shows only 5 to 10 per cent loss in color value. The greater the excess of urea added, the slower the rate of fading. For instance, the color produced when 0.5 cc. of hyprobromite is employed, fades in 2 to 3 minutes. If 0.5 cc. of 20 per cent urea is added, the color fades entirely in 20 to 30 minutes, but with 1 cc. of 40 per cent urea, the color does not entirely fade, even after 24 hours. The presence of urea does not completely prevent fading

but does slow the rate of fading sufficiently so that if colorimetric readings are made within 5 minutes, accurate comparisons can be made.

TABLE II.

Effect of Certain Compounds on the Color Production Given by Arginine.

Substance.	Amount added.	Arginine.	Color developed.
	mg. per 5 cc.	*mg. per 5 cc.*	*per cent*
Ammonium sulfate.*	0.1	0.01	45.5
	0.1	0.02	46.5
	0.05	0.02	71.5
	0.05	0.01	71.0
	0.02	0.02	91.0
	0.01	0.02	98.5
Urea.	4.0	0.02	52.6
	2.0	0.02	76.1
	2.0	0.04	76.1
	1.0	0.02	87.0
	0.5	0.02	100.0
Histidine dihydrochloride.	0.2	0.02	76.1
	0.2	0.04	78.1
	0.1	0.01	94.3
	0.1	0.02	95.2
	0.05	0.02	100.0
Creatine.	1.0	0.02	88.0
	0.5	0.01	100.0
	0.5	0.02	100.0
Creatinine.	2.0	0.01	100.0
	2.0	0.02	100.0
Uric acid.	0.2	0.02	100.0
	0.1	0.02	100.0
Glucose.	10.0	0.02	100.0
Glycerol.	20.0	0.02	100.0
	40.0	0.02	100.0
	60.0	0.02	90.0
Lysine monohydrochloride.	0.5	0.02	100.0
		0.04	100.0
Tryptophane.	0.1	0.02	100.0
Glycine.	1.0	0.02	100.0
Alanine.	1.0	0.02	100.0

* Ammonium sulfate values are given as the quantity of ammonium nitrogen present. Values for the other compounds are given as the weight of the compound actually employed.

It is necessary that the reaction be carried out at about 4°, because, at ordinary temperatures, the reactivity of the hypobro-

mite is so great that destruction of the guanidine group and of the color produced is very rapid, less color being obtained. The quantities of arginine that have been used vary between 0.005 mg. and 0.05 mg. per 5 cc. The volumes of the standard and the unknown must be the same since less color is developed with the same quantity of arginine, the greater the volume of solution. However, after the color has been developed it may be diluted with water.

The arginine content of solutions, which were made up by another member of the laboratory staff, was determined. Table I shows the results of individual determinations and shows the possibilities of this reaction as a colorimetric procedure for arginine.

Colorimetric determinations are influenced by the presence of other substances that occur in biological material. The reaction for arginine is profoundly influenced by the presence of certain substances, if they occur in sufficient concentration to render even a qualitative test of no value. Ammonia, histidine, tyrosine, and tryptophane produce a marked interference and, to a lesser degree, creatine and urea. Thus, the presence of either 0.3 mg. of ammonia nitrogen, 0.6 mg. of histidine dihydrochloride, 0.3 mg. of tyrosine, 0.3 mg. of tryptophane, 2.0 mg. of creatine, or 8.0 mg. of urea in 5 cc. with 0.01 to 0.04 mg. of arginine, either prevents all color formation or the color is so altered that even for qualitative purposes the test is worthless. With the exception of sulfides, sulfites, and iodides, no salts have been found which influence the color produced by arginine. Obviously metals forming insoluble hydroxides must be removed before the test can be performed.

Table II shows the amount of color developed by arginine in the presence of various compounds. Other compounds will probably be found that interfere, when the test is applied to various biological fluids. However, sufficient evidence has been presented to show the possibilities of this reaction as a method for the colorimetric determination of arginine.

CONCLUSION.

A modified Sakaguchi reaction is described which can be employed for the colorimetric determination of arginine.

BIBLIOGRAPHY.

Poller, K., *Ber. chem. Ges.*, **59**, 1927 (1926).
Sakaguchi, S., *J. Biochem.*, Japan, **5**, 25 (1925).

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