CLXXVII. THE USE OF THE SAKAGUCHI REACTION FOR THE QUANTITATIVE DETERMINATION OF ARGININE.

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The determination of this amino-acid has hitherto been based on the formation of urea or ammonia from it, either by boiling with 20 % potassium hydroxide solution, or under the influence of arginase. The first qualitative test for arginine, the diacetyl reaction of Harden and Norris [1911], has only recently been applied to its colorimetric estimation [Lang, 1932]. Sakaguchi's reaction for arginine [1925] is not only specific and very sensitive, but is also applicable to quantitative analysis. As the determination of arginine by Van Slyke's method demands a considerable amount of material, colour tests for small quantities of arginine would be very useful. As shown by Weber [1930], the Sakaguchi reaction can be used for the quantitative determination of 0·05–0·005 mg. arginine. Several precautions must however be taken.

In analysing some blood-proteins by the Van Slyke procedure, we used this reaction and found it reliable. The instructions given by Weber were at first followed. Instead of a colorimeter, a Zeiss step-photometer was used. This made a more accurate study of the colour development possible, and some valuable information was gained.

The procedure recommended by Weber was as follows. To 5 cc. of a solution containing 0·05–0·005 mg. arginine, 1 cc. 10 % sodium hydroxide solution was added, and 1 cc. of a 0·02 % solution of α-naphthol. After cooling on ice, 0·2 cc. of a hypobromite solution was added, and 4–6 seconds later, 1 cc. of a 40 % urea solution. The colorimetric reading should be made within 5 minutes. In the presence of α-naphthol and hypobromite a reddish colour is given by arginine and other mono-substituted guanidines. The colour is not given by any other amino-acids.

The colour is very sensitive to an excess of hypobromite, and therefore as soon as it has developed, urea is added in order to destroy the excess of hypobromite. Furthermore, as pointed out by Weber, the maximum colour is obtained with a certain amount of hypobromite only. This is to be considered in preparing the hypobromite solution and in performing the test on solutions which react with hypobromite. According to Weber the optimum

amount of hypobromite to be added is to be ascertained in each individual case. The hypobromite solution is prepared by dissolving 2 g. of bromine in 100 cc. of 5 % cooled sodium hydroxide solution. More or less bromine (3·5 or 1·6 g. bromine in 100 cc.) gives a fainter coloration.

The colour is greatly influenced by the temperature and if the reagents are not cooled, a much fainter colour is obtained. The test-tubes containing the mixed solutions are therefore kept for at least an hour plunged in ice before the addition of the hypobromite. The urea solution is cooled in the same way.

It takes about 10 seconds for the colour development to reach its maximum and the urea should not be added until this has been attained. Thus, in two series, a 12 % higher colour intensity was obtained when the urea solution was added after 10 seconds instead of after 5, as recommended by Weber. After reaching its maximum, the colour begins to fade about 20 seconds after the hypobromite solution has been added. The cooled urea solution is therefore to be added exactly 15 seconds after the hypobromite. Once stabilised, the colour remains constant for 6–7 minutes. This is important for the photometric reading. If the urea solution has not been cooled, the colour begins to fade in 4–5 minutes.

Owing to the lability of the colour, quite the same accuracy cannot be obtained by this reaction as by the diazo-reaction for histidine. The colour is not proportional to the amount of arginine present in the tests. With smaller amounts of arginine, a relatively greater colour intensity is obtained. Thus *e.g.* the coefficient of extinction $\epsilon\left(-\log_{10}\frac{I_p}{I_a}\right)$ calculated for 0·1 mg. arginine (*d* being 1 cm.), was 1·73 with 0·04 mg. arginine and 1·63 with 0·07 mg. The colour can thus be used for colorimetric purposes only if the standard is very close to the unknown. As this relative increase in colour intensity with decreasing amounts of substance is quite regular, a photometer can very well be used. The photometer scale is plotted on semilogarithmic paper. The amounts of arginine taken in a standard series are used as abscissae and the photometer scale as the logarithmic ordinates. The points fall on a straight line, and thus no further calculation is required. If the cooling is not sufficient, another line may be obtained. It is advisable each time to have controls of different concentrations in order to check this.

The solutions used are those of Weber.

1. An arginine solution containing 0·04–0·07 mg. arginine in 5 cc.
2. A 10 % solution of sodium hydroxide *e natrio*.
3. A 0·02 % solution of α-naphthol, made fresh each time by the dilution of 20 cc. of a stock solution (0·1 % in alcohol) to 100 cc. with water.
4. A hypobromite solution made by dissolving 2·5 g. (0·46 cc.) bromine in 100 cc. 5 % cooled sodium hydroxide solution. This solution keeps for months in the cold.
5. A 40 % urea solution.

Procedure. A test-tube, 150 × 18 mm., with 5 cc. arginine solution is cooled on ice. 1 cc. of sol. 2 (sodium hydroxide solution) is added and 1 cc. of sol. 3 (α-naphthol), the solutions mixed and the test-tube well embedded in ice. After a time (1 hour) 0·2 cc. of sol. 4 (hypobromite) is added with vigorous shaking, and exactly 15 seconds later 1 cc. of sol. 4 (cooled urea solution). The photometric reading is made against water in S. 50 within 6–8 minutes. For amounts of arginine between 0·04 and 0·08 mg., which is the range found most convenient in our experiments, a 0·5 cm. dish is used. The amount of arginine present is found directly from the curve on semi-logarithmic paper.

If all precautions are strictly taken, this method is as good as any ordinary colorimetric procedure. The duplicates agree to within 1–2 %. As neutralised phosphotungstic acid up to 1 % of the final arginine solution does not influence the colour intensity this colorimetric method may replace the alkaline hydrolysis of the Van Slyke procedure.

In analysing the basic fraction of the amino-acids of haemoglobin after dissolving the phosphotungstic precipitate in alkali, for histidine with the diazo-reaction of Pauly and for arginine with this method, we found that the figures agreed exactly with those found by Vickery and Leavenworth with their isolation method. (See the preceding paper.)

Summary.

The reaction of Sakaguchi with α-naphthol and hypobromite has been used for the determination of arginine in protein hydrolysates. The details of the colorimetric procedure have been studied. If the necessary precautions are taken, the reaction can be used for quantitative purposes. It is also applicable to the determination of the arginine content of the basic fraction of the amino-acids in the Van Slyke procedure.

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