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Determination of α-Alanine by Ninhydrin Oxidation

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Virtanen and collaborators [1-3] have in this laboratory worked out a method for determination of certain amino acids by ninhydrin oxidation. In this method the amino acids are converted to aldehydes with one less carbon atom ($R \cdot CH(NH_2) \cdot COOH \rightarrow R \cdot CHO$); the volatile aldehydes formed are distilled into a bisulfite solution, and their total amount is determined by means of iodometric titration. The group of amino acids which can be determined in this way, the so-called »volatile aldehyde amino acids», is comprised by α-alanine, valine, leucine, isoleucine and possible other isomers, phenylalanine, and methionine.

It has been shown in recent years that α-alanine has some special importance in the metabolism of amino acids and proteins. It is one of the amino acids active in the transamination reaction, and investigations concerning the protein synthesis in yeast (Roine [4]) showed that alanine together with the dicarboxylic amino acids is abundantly formed in the very beginning of the nitrogen uptake by yeast. Alanine, thus, occupies a special position in the group of »volatile aldehyde amino acids», and a separate determination of it seems to be important in many cases.

As reported earlier by one of us [4] alanine can be determined conveniently in connection with the determination of »volatile aldehyde amino acids» by means of some colour reaction, specific for acetaldehyde. The blue colour which acetaldehyde gives with sodium nitroprusside and piperazine is well suited for this purpose. The solution, the alanine content of which is to be analyzed, is first extracted with ether to remove acetaldehyde, pyruvic acid, and other possibly disturbing substances. After the oxidation with ninhydrin, distillation and titration, acetaldehyde is determined photometrically in a way similar to that used by Fromageot and Heitz [5].

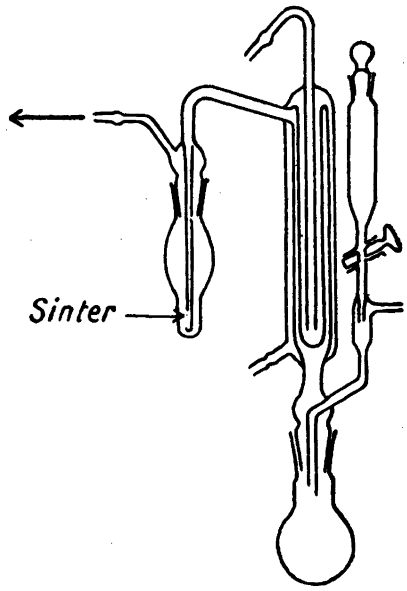

Fig. 1. Lieb-Zacherl apparatus.

PROCEDURE

A sample of the solution to be analyzed, the alanine content of which corresponds to 0.5—20 mg, is made acid with sulphuric acid and extracted over night with aldehyde-free ether in a Partheil-Rose extractor [6]. The excess of sulphuric acid is neutralized with sodium hydroxide, the remaining ether evaporated on a water bath at 60—70° C, and the volume of the residue made up to 20 ml. 2—10 ml of this solution are placed in the reaction flask of a Lieb-Zacherl apparatus (Fig. 1), made up to 10 ml and 1 g of potassium dihydrogen phosphate, and 2.1 g of sodium chloride are added. The receiver is charged with 5 ml of 1 % sodium bisulphite solution. The solution in the reaction flask is brought to boil, whereupon 3 ml of an 1 % ninhydrin solution are introduced through the side tube. The distillation is continued for 60 min after which the receiver is disconnected, the tube with sintered end washed twice with small amounts of water, and the excess of bisulphite destroyed with 0.1 *N* iodine solution. After the addition of starch the solution is titrated colourless with 0.01 *N* sodium tiosulphate. 10 ml of a saturated sodium bicarbonate solution are added and the bisulphite thus liberated, titrated with 0.01 *N* iodine. After the addition of some drops of 0.01 *N* sodium tiosulphate, the titrated solution is placed in a 50 ml volumetric flask and filled to the mark.

A 6 ml aliquot of the solution is placed in a 10 ml measuring cylinder, 0.5 ml of 4 % sodium nitroprusside solution and 1.5 ml of 25 % (saturated)

piperazine solution are added, mixed thoroughly and placed in a 2 cm cell of Pulfrich photometer (of the two piperazine preparations tried by us, only that of Schering A. G. (Berlin) gave reliable results). The colour reaches its maximum intensity in some 2 or 3 min and remains there for 1—2 min during which time the readings are to be made, using colour filter S 57. The amount of alanine equivalent to the reading is read from a calibration curve constructed with pure acetaldehyde.

DISCUSSION

In addition to acetaldehyde only acrolein and propionaldehyde give the same colour reaction with sodium nitroprusside and piperazine, the intensity of the colour, however, in the latter cases being very weak. The aldehydes which are formed of valine, leucine, isoleucine and possible other isomers, phenylalanine, and methionine, do not give this reaction. Under the conditions described above, the method is thus specific for alanine. The smallest amount of alanine which can be reliably determined with the given procedure, is about 0.5 mg.

The procedure is rapid and simple and is especially well suited to be performed in connection with the determination of »volatile aldehyde amino acids» (*cf.* Virtanen and Rautanen [3]). In this laboratory the method has been used for the determination of alanine in yeast [4] and plant [7] extracts as well as in protein hydrolysates.

SUMMARY

A micro method for determination of α-alanine is described. Alanine is oxidized with ninhydrin and the acetaldehyde formed is determined photometrically using the specific colour reaction which it gives with sodium nitroprusside and piperazine.

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