114. *Purified Diastases.*

IV. *Composition and Constitution of Diastase.*

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The mould diastase (Lintner's value=600000) and the malt diastase (Lintner's value=150000) purified by the author's method, gave always the typical pentose reaction, though they did not reduce Fehling's solution.

On hydrolysing these samples with 1-2% sulphuric acid or hydrochloric acid for one hour, however, strong reduction of Fehling's solution appeared. From the hydrolysed solution, the insoluble residue was removed by filtration and the agricones which contained nitrogen and were dissolved in solution, were also removed by adding 4-5 times of acetone or alcohol. After the filtration of agricone the filtrates therefrom were neutralised with barium hydroxide and further concentrated in vacuum.

34-38% pentose was estimated by the quantitative analysis according to Bertrand, and this pentose was further determined as ribose on account of *p*-brom-phenylhydrazone which recrystallized from hot water in plate or small needle-like forms with a little brownish yellow colour, melting at 160-162°C, containing 10-11% nitrogen.

While mould diastase and malt diastase gave the typical pentose reaction, purified bacterial diastase (Lintner's value=50000) neither reduced Fehling's solution, nor showed pentose reaction. However, on hydrolysing bacterial diastase with 1-2% sulphuric acid for one hour, a strong reducing power was detected, and the sugar was assumed to be hexose.

32-35% hexose was estimated as glucose by Bertrand's method, the assumption of glucose being made on account of phenylhydrazone and osazone. The osazone recrystallized from acetone, melted at 180°C and contained 14-15% nitrogen.

It is one of the most important facts that there exist two kinds of diastase, i.e., "Pentosic diastase" and "Hexosic diastase" both containing almost the same amounts of sugars.

The purified diastases all contained 11-12% nitrogen, which gave the reactions of amino nitrogen and no reaction of ammonia nitrogen.

On filtering the solution of diastase hydrolysed with 1-2% sulphuric acid and further on adding 4-5 times of acetone to the filtrate, the agricones were precipitated completely, leaving sugars in the solution.

Thus, we see that the constitutions of diastases may be assumed as follows:

Furthermore, the agricones of the three kinds of diastase showed almost similar behaviors, physical as well as chemical. All the three agricones gave ninhydrine reaction, but no Nestler's reaction.

The agricones of mould diastase and malt diastase also gave biuret reaction though that of the bacterial diastase was not quite clear as to this reaction.

While diastases were precipitated easily by acids, it was not the case with agricones. When diastases were heated with acid, it used to be thought as precipitated out of the solution, though the agricones always remained in solution. Moreover, diastase could be salted out with ammonium sulphate, while on the other hand, agricones failed to be precipitated by the same salt. It is obvious from these properties, that agricones behaved exactly as an organic compounds of fairly small molecular weight.

After the agricone of mould diastase was hydrolysed with 25% sulphuric acid for 10 hours, sulphuric acid was removed by adding barium hydroxide. On concentrating this filtrate, a great deal of crystals was obtained. When the crystal was washed with a little water and recrystallized out of hot water, a needle-shaped, silky lustrous crystal was obtained, which gave Millon's, diazo and ninhydrine reactions and which contained 8% nitrogen. This crystal was affirmed as tyrosine ($C_9H_{11}O_3N$)

Tyrosine was also isolated from the agricones of malt diastase and of bacterial diastase, by the same operation as above.

The experimental results led the author to believe that tyrosine was one of the important components of all the three diastases.

The filtrates above mentioned were further treated with phosphotungstic acid, thereby a great deal of precipitate was obtained containing basic amino acid. It is noteworthy that before decomposition, diastase and agricone were equally precipitable by the same reagent.

The precipitate was treated with barium hydroxide and after filtration, the excess barium hydroxide was removed with sulphuric acid. The filtrate thus obtained gave ninhydrine reaction and Sakaguchi's reaction.

On treating the filtrate with flavianic acid an insoluble brownish precipitate was obtained and after recrystallization from hot water, a typical plate-formed crystal of flavianate was found, containing 17%

nitrogen. The crystal was brownish coloured at 250° C. It is clear that the crystal was nothing but arginine flavianate.

The author further succeeded in isolating arginine also from other two agricones by the similar method. Like tyrosine, arginine was also assumed to be one of the important components of agricones.

Finally, the filtrate of phosphotangstic acid was treated with barium hydroxide in excess, and after removing the precipitate, the filtrate was neutralized with sulphuric acid.

On filtering the solution and concentrating the clear filtrate, a plate-formed crystal was obtained, which was recrystallized by dissolving it in cold water, removing the insoluble residue and concentrating the solution.

The recrystallized crystal gave the typical ninhydrine reaction but no Millon's or Sakaguchi's reaction. Furthermore, a peculiar smell of isoamylamine was detected by heating with soda lime in a tube.

The copper salt of this crystal was a typical plate-formed, faintly blue-coloured crystal, containing 7~8% nitrogen.

From this fact, it was decided to be leucine ($\text{C}_6\text{H}_{13}\text{O}_2\text{N}$)

The author also isolated leucine similarly from other agricones in the same way, and came to believe that leucine, like tyrosine and arginine, was one of the important components.

In spite of the author's endeavour no more amino acid was obtained from the decomposed products of agricones.

These experimental results led the author to believe that the agricones of the three diastases consisted of the same components, i.e., one molecule each by rough estimation of tyrosine, arginine and leucine, i.e.

The author is now occupied in further studying the arrangement of these components in each agricone of different diastases.

The method for the isolation of the components above mentioned may be shown briefly in the following scheme:—

Thus, the author arrived at the conclusion that diastases prepared from different sources have similar agricones of small molecular weight, namely 432, containing one molecule each of tyrosine, arginine, leucine, and two molecules of sugars, i e.

The molecular weights of diastases obtained by the Beckmann's freezing-point method were 760-790, and the calculated molecular weights were 696 for the pentosic diastases, and 732 for the hexosic diastases.

Summary.

In the investigation reported in this series of papers the author succeeded in obtaining satisfactory purification of diastases from three different sources, mould, malt and bacteria. Numerous experiments carried out with the purified diastase, brought out several points in its alleged properties that must be revised, but by far the most important fact discovered was that diastases are saccharides, not proteins. The component agrigone consists of tyrosine, arginine and leucine in all diastases, while sugar may be either pentose (in mould and malt diastases) or hexose (in bacteria diastase), thus giving two kinds of diastases, pentosic and hexosic. The molecular weights of diastases were estimated to be 696 and 732.