CXLI. A RAPID COLORIMETRIC METHOD OF ESTIMATING PENTOSEs.

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THE basis of the estimations of pentoses now in use is the quantitative conversion of the pentose into furfural by boiling with dilute hydrochloric acid. The furfural so formed, being volatile in steam, passes over into the distillate (together with water and HCl), and is then estimated by one of several methods: Plimmer [1920]; Ling and Nanji [1921]; Youngburg and Pucher [1924]. While the method herein described also depends upon the estimation of furfural, it differs from the others in certain respects which make it very rapid and easy to execute.

Youngburg and Pucher [1924] have described a colorimetric method of estimating the furfural in the distillate which depends upon the well-known colour given by furfural in the presence of aniline acetate. Benzidine has now been found to give a much more intense colour with furfural than aniline, and (in my opinion) a colour much more easy to match against a standard. The colour given by benzidine is a deep crimson violet. It is, however, completely destroyed by water. This difficulty can easily be overcome by shaking the "unknown" furfural solution with benzene, in which furfural is much more soluble than in water, and, after partition has taken place, using the benzene portion for the furfural estimation. The "standard" solution of furfural in water must also be shaken with benzene under identical conditions. The colours are best matched when the "unknown" and "standard" are within 25 % of each other, but a rough idea of the strength of the "unknown" can be obtained over a much wider range. The reason for this is that the reagent (described below) is itself a light yellow and this makes the weaker solution appear more red and less violet than the stronger, because in the former one is looking through a deeper layer of the yellow reagent. This micro-method of estimating furfural is obviously capable of general application.

By taking advantage of the fact that furfural is freely soluble in benzene a very simple, easy method of estimating the pentoses has been devised (it has, however, some well-defined limitations). This involves no distillation, and requires only test-tubes and a water-bath in the way of apparatus.

Method.

The "standard" is made up as follows: 3 cc. 0.1 % arabinose, 3 cc. H_2O and 3 cc. conc. HCl are placed in a test-tube. 1 cc., 2 cc. or 3 cc. etc. of the "unknown" solution is placed in another test-tube and the volume made up to 6 cc. with water; 3 cc. of conc. HCl are then added. The tubes are placed in a water-bath at 100° for 2 hours. Each test-tube is closed by a cork through which passes a long piece of glass tubing which acts as a condenser. After 2 hours the tubes are removed from the bath and cooled, any drop in the glass tube being blown out. 4 cc. of benzene are added to each tube which is then shaken vigorously for 3 minutes. The test-tubes are then allowed to stand 30 minutes. There may be some black material in the layer of benzene above the "unknown," but this can be dispersed by spinning the tube between the hands when the black material sinks or sticks to the side. 2 cc. of the benzene layer are then withdrawn with a pipette and placed in a dry test-tube; it is essential that none of the aqueous layer be withdrawn with the benzene. 4 cc. of benzidine reagent are then added to each of the test-tubes. The colour takes some 15 minutes to develop, and the tubes may be matched any time between 30 minutes and 2 hours after adding the reagent. It is best to have each tube in duplicate. The duplicates may conveniently be mixed just before matching in the colorimeter. The number of estimations which can be carried out simultaneously is only limited by the space available for test-tubes in the water-bath.

This method is only capable of application (a) when there is no colouring matter extracted by the benzene from the "unknown," or at any rate not enough to interfere with the colorimetric estimation of the furfural; (b) when the pentose to be estimated is the same as the "standard," because all the pentose is not quantitatively converted to furfural by this method, and as different pentoses (and glycuronic acid) may be converted to furfural at different rates, equilibrium may not have been reached in all cases; (c) when the "unknown" does not contain enough proteins or buffering substances to decrease appreciably the concentration of HCl . This must be a matter for experiment in each case.

The benzidine reagent is made up as follows: 0.5 g. of benzidine is dissolved in 100 cc. of equal parts of absolute alcohol and glacial acetic acid. The solution is then filtered. It keeps for some time but it is best to make it up fresh once a week.

Some experimental results on a urine to which known amounts of arabinose had been added may now be given. The sample of urine used contained by this method 0.02 % of "pentose." Actually this substance was probably a mixture of pentoses and glycuronates, and to estimate its absolute amount one would have to distil the urine with HCl and estimate the furfural in the distillate. Solutions of arabinose in urine of the following strengths were then made up: (a) 1 %; (b) 0.4 %; (c) 0.1 %; (d) 0.05 %. 0.3 cc. of (a), 0.8 cc. of (b),

3.0 cc. of (c), 6.0 cc. of (d) were taken for each estimation, the "standard" being 3 cc. of 0.1 % arabinose, 3 cc. H₂O and 3 cc. conc. HCl. With the "standard" set at 20 the colorimeter readings were (a) 19.1; (b) 17.3; (c) 16; (d) 14.9, making the strength of arabinose by estimation:

$$(a) 1.05 - 0.02 = 1.03 \%$$

$$(b) 0.43 - 0.02 = 0.41 \%$$

$$(c) 0.125 - 0.02 = 0.105 \%$$

$$(d) 0.067 - 0.02 = 0.047 \%$$

SUMMARY.

A rapid and simple method of estimating pentoses is described involving no distillation and depending on a colour reaction between furfural and benzidine.

REFERENCES.

- Ling and Nanji (1921). *Biochem. J.* 15, 466.
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Youngburg and Pucher (1924). *J. Biol. Chem.* 61, 741.