STUDIES ON PENTOSE METABOLISM.

II. A MICRO METHOD FOR THE DETERMINATION OF PENTOSES AND PENTOSANS.

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The decomposition of pentoses or pentosans by means of hydrochloric acid has long been the most extensively used method for transforming these substances into furfural. The subsequent determination of the furfural has been done mainly by precipitating the furfural as phloroglucide and weighing the product according to the directions of Kröber, as modified by Tollens, and at present the method of the Association of Official Agricultural Chemists (1). For the literature on this subject the papers of Pervier and Gortner (2) are referred to.[1]

The fact that the above method is not adaptable as a micro method, is very time-consuming, yields a phloroglucide of inconstant composition (4), as well as that it is not specific for furfural (5), has made it inefficient for many determinations, particularly those of a biological nature where only small amounts of material or those low in pentose percentage are in question.

Since the development of the steam distillation procedure by Pervier and Gortner and by work in this laboratory, for transforming pentoses into furfural and the colorimetric method of Youngburg and Pucher (6) for the determination of furfural, it seemed that it would be desirable if in the distillation the volatile hydrochloric acid could be replaced by some other reagent which would not itself appear in the distillate, thus obtaining the furfural in a

[1] The colorimetric method of McCance (3) appeared after the work of this paper was completed. The knowledge that refluxing with strong acid such as HCl destroys some furfural must make the method of McCance an approximate one only.

smaller volume, devoid of acidity, and therefore suitable for the direct colorimetric determination.

With this object in view the writer has searched for a suitable reagent to replace the HCl in distilling. The literature shows that the following reagents have been employed at one time or another for the decomposition of pentoses into furfural: pyrolusite and sulfuric acid, sulfuric acid, solution of zinc chloride, glacial acetic acid, phosphoric acid, and hydrochloric acid. Only the last seems to have stood the test of time in this respect and its superiority has seemed apparent. The writer, however, has tried all of the above and in addition has used glycerol. The results obtained have eliminated all except phosphoric acid as a competitor of hydrochloric acid for use in this method.

Mann, Kruger, and Tollens (7) in 1896 reported the use of phosphoric acid as follows: "Ebensowenig Erfolg hatten wir, als wir Xylose statt mit Salzsäure mit Phosphorsäure destillierten, den es bildete sich erheblich Humin, und das enstandene Furfural betrug nicht mehr, sondern etwas weniger als sich mit Salzsäure bildet. (Sehe das Nähere in der Dissertation.)"

The Dissertation of Dr. Kruger is not available. With the use of steam distillation the phosphoric acid method of decomposition might obviously give more satisfactory results because there is efficient means for removing the furfural from the destructive effect of the strong acid. This has been found to be correct and it also has been found that phosphoric acid liberates furfural more rapidly than does hydrochloric acid! There is no loss of furfural, recovery being 100 per cent upon a pure furfural solution. The experimental conditions have been worked out by which a maximum yield of furfural is obtained and are implied in the method which follows.

Method.

Special reagents required are:

1. Furfural.—Distil a good grade of furfural, *e.g.* Pfanstiehl, at a pressure of 20 to 30 mm. For most practical work the product from distillation at atmospheric pressure is suitable.

2. Furfural Standard.—Dilute 1 cc. of the above furfural up to 500 cc., and dilute 1 cc. of this solution up to 232 cc. 1 cc. = 0.01 mg. of furfural.

3. Redistilled Aniline.—It is sufficient to distil under atmospheric pressure, but under reduced pressure an absolutely colorless product is obtained.

The apparatus used is shown in Fig. 1. It is essentially a test-tube *B* (25 × 150 mm.) containing the reaction mixture and is connected with a steam generator *A* and a condenser. A convenient form of steam generator is a 250 cc. three-necked Pyrex flask, although any other form will do. A small condenser with a water jacket about 6 inches long is convenient, although a larger one will serve the purpose.

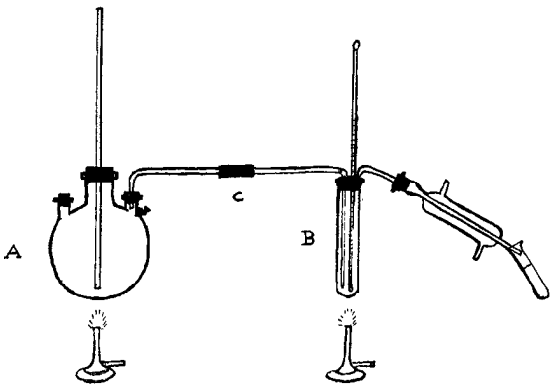

FIG. 1.

Procedure.

Place a quantity of the substance to be determined, calculated to contain between 0.1 and 1.0 mg. of pentose or pentosan, in the test-tube; add about 3 cc. of 85 per cent phosphoric acid and mix. Start the steam generator but do not yet connect at *c*. Connect the test-tube with the condenser and heat the tube with a micro burner until the reaction mixture reaches about 125°. This implies that some water is first distilled off, should much have been introduced with the pentose material. Connect at *c*, causing the influx of a slow current of steam, and distil at about 175° (not exceeding 180°) into test-tube receivers graduated at 10 cc. As each distillate is obtained, transfer exactly 2 cc. to another test-tube containing 0.25 cc. of aniline; add 2 cc. of glacial acetic

acid and set aside for 30 seconds for color development. When no color develops in subsequent tubes, cease distillation and consolidate 2 cc. (or more) aliquots from each of the distillates which give color.

Determine furfural colorimetrically in the consolidated aliquots as follows: To one 10 cc. graduated tube add 5 cc. of the consolidated distillate and to another similar tube 5 cc. of the furfural standard (0.05 mg. of furfural). Add to each tube 0.5 cc. of aniline and 4 cc. of glacial acetic acid. Fill to the mark with water and set aside in a dark or semidark place for 15 minutes. Read in a colorimeter. Readings must not extend over 40 minutes from the time of mixing the reagents.

If the color of the unknown is too deep it may be diluted with a mixture of aniline and acetic acid in the above proportions.

The calculation of furfural will of course depend on the number of distillates consolidated. From the furfural obtained is calculated the pentose yield using the following conversion factors:

As *d*-xylose:	furfural	found	× 1.56
" *d*-ribose:	"	"	× 2.00
" *l*-arabinose (or *d*-arabinose):	"	"	× 2.40
" *d*-lyxose:	"	"	× 3.00

In the determination of pentosans the writer believes that the results should be expressed as pentose, the particular pentose depending on the pentosan material source.

DISCUSSION.

The literature on the yield of furfural from pentoses by the use of various reagents already indicated in this paper, particularly by hydrochloric acid, gives varying results for *d*-xylose and for *l*-arabinose, and no figures have been published for *d*-ribose and *d*-lyxose, the remaining members of the naturally occurring pentose group of carbohydrates. Pervier and Gortner in their Table I (2) show that pentose recovery by various investigators on xylose ranges from 87.5 to 100.8 per cent, and on arabinose from 73.4 to 100.8 per cent. This is an unusual variation in analytical results and points to some variable which needs better control. That this variable is the partial destruction of furfural by strong

acid during decomposition of the pentose has lately been elucidated by Pervier and Gortner. They obviate this by removing the furfural as soon as formed, by a current of steam.

It is well known, however, that all of the pentoses are not converted into the theoretical yield of furfural. This is firmly established since the destruction of the furfural formed can now be eliminated by steam distillation. Numerous experiments have been made by the writer to find the conditions under which maximal amounts of furfural are produced from the pentoses. Such yields are shown in Table I and were obtained by the method described in this paper.

Thus only *d*-xylose is fully converted into furfural; *d*-ribose,

TABLE I.

Showing Furfural Yield or Pentose Recovery by Phosphoric Acid-Colorimetric Method.

Pentose.	Furfural yield or pentose recovery.
	per cent of theoretical
d-Xylose*	100.0
d-Ribose	78.5
l-Arabinose and *d*-arabinose	64.9
d-Lyxose	52.2

* Until recently known as *l*-xylose. See Armstrong, E. F., Monograph on biochemistry, The carbohydrates and the glucosides, London and New York, 4th edition, 1926, 38.

l- and *d*-arabinose, and *d*-lyxose follow in decreasing amounts of furfural yield. While the recovery for *d*-xylose is 100 per cent of the theoretical value, the figure for *l*-arabinose, 64.9, is less than that found by others (73.4 to 100.8) by the hydrochloric acid method. As previously mentioned, recovery figures for *d*-ribose and *d*-lyxose as well as for *d*-arabinose, have not been published heretofore. It is interesting to find that *d*-arabinose yields the same amount of furfural as *l*-arabinose. At least in this case of enantiomorphic forms the difference in configuration does not affect the furfural yield. This is of some importance because the arabinose in the urine of pentosurians has been found by Neuberg (8) to be the racemic form. In its determination, then, no error will be made because the racemic form is equivalent

TABLE II.

Showing Yield of Furfural from Various Substances by Micro Method.

Substance.*	Furfural yield.
	per cent
Carbohydrates:	
Maltose	5.71
Dextrose	4.84
Dextrin	3.93
Sucrose	3.52
Inulin	1.98
Starch (potato)	1.85
Glycogen	1.51
Galactose	1.38
Levulose	1.12
Lactose	1.10
Other substances:	
Albumin, egg	0.08
" serum	0.04
Cholesterol	None.
Creatinine	"
Cystine	Trace.
Casein	0.04
Fibrin	0.05
Glutamic acid	None.
Glycuronic acid	About 10.0
Glycocoll	None.
Gelatin	0.02
Gum arabic	8.51
Hemoglobin	0.02
Inosite	None.
Mucic acid	0.10
Nucleic acid (from yeast)	10.40
Ouabain (a rhamnose glucoside)	None.
Rhamnose	"
Salicin (glucose-*o*-oxybenzyl alcohol)	2.75
Tyrosine	Trace.
Dulcitol	None.
Mannitol	"
Sorbitol	"

* The purest substances obtainable were used. In a number of cases purifications were made in this laboratory.

to a mixture of *d* and *l* forms and they yield equal amounts of furfural.

It is well known that substances other than pentoses, particularly glycuronic acid, yield furfural when heated with strong acid. The yields from various carbohydrates and from a number of miscellaneous substances by the phosphoric acid-colorimetric method herein described have been determined and are given in Table II.

It can be seen that, of the common carbohydrates, maltose yields the greatest amount of furfural, 5.71 per cent. Lactose yields the least, 1.1 per cent. All yields are appreciable. Rhamnose, a methyl pentose, yields no furfural. Pure glycuronic acid was not available but glycuronic acid esters were extracted from urine according to the method of Neuberg and Schewket (9) and yielded much furfural. Dr. Pucher of this laboratory has found a yield of about 10 per cent from glycuronic acid by the phosphoric acid-colorimetric method. Mann, Kruger, and Tollens (7) found 17.27 per cent by the hydrochloric acid distillation method after checking up reports of 46 per cent by Gunther and 31.4 per cent by de Chalmot.

Substances such as amino acids, proteins, nucleic acid, creatinine, etc., were determined in order to test the availability of the method for the determination of pentose in blood, tissues, urine, etc. From the data obtained it appears that only substances containing a carbohydrate unit in the molecule, excepting methyl pentoses like rhamnose, and glycuronic acid, may yield appreciable amounts of furfural. This is in accord with data in the literature.

In determining pentoses by furfural yield, then, one must take into account the probable presence of interfering substances. Body fluids may yield appreciable furfural from non-pentose sources, such as from glycuronic acid, glycogen, dextrose, and nucleic acid compounds. Under present circumstances, however, the method may be of value in following the course of the metabolism of substances which are predominatingly furfural-yielding.

SUMMARY.

Pentoses may be determined in a micro way by distillation with phosphoric acid and steam. The furfural formed is determined colorimetrically.

Phosphoric acid liberates furfural more rapidly from pentose material than does hydrochloric acid. In general, however, the yield of furfural is not greater than with hydrochloric acid.

The method may be of value in metabolism work and for other purposes.

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