S 31. *A Micro-method for the Determination of Pentoses by Photoelectric Spectrophotometry.*

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A method for the spectrophotometric determination of pentoses on a micro-scale has been developed. It is based on the dehydration of the sugar by 85% phosphoric acid at 170°, the removal of the furfuraldehyde so formed by steam-distillation, and the subsequent determination of this by means of its light absorption at 2785 A. Errors involved are less than 2% for amounts of pentose between 0·5 and 2 mg., but a successful method for the determination of pentoses in the presence of other sugars has not yet been achieved.

Almost all the methods that are available for the determination of pentoses depend upon the conversion of these sugars into the volatile compound furfuraldehyde, which can be distilled off and determined either gravimetrically, volumetrically, or colorimetrically.

In the gravimetric methods the furfuraldehyde may be condensed with phloroglucinol (Schorger, *Ind. Eng. Chem.*, 1928, **15**, 748; Dorée, " Methods of Cellulose Chemistry," Chapman and Hall, London, 2nd Edition, 1947, p. 381) or with either barbituric acid or thiobarbituric acid, the precipitates being collected and weighed in each case (Dox and Plaisance, *J. Amer. Chem. Soc.*, 1916, **38**, 2156; Campbell and Smith, *Biochem. J.*, 1937, **31**, 535; Lechner and Illig, *Biochem. Z.*, 1938, **299**, 174; Jayme and Sarten, *Biochem. Z.*, 1942, **312**, 78).

Of the volumetric methods probably the one most thoroughly investigated depends upon the absorption of bromine liberated from an excess of potassium bromide–bromate mixture (Pervier and Görtner, *Ind. Eng. Chem.*, 1923, **15**, 1255; Powell and Whittaker, *J. Soc. Chem. Ind.*, 1924, **43**, 35T; Hughes and Acree, *Ind. Eng. Chem. Anal.*, 1934, **6**, 123; 1937, **9**, 318; Dorée, *op. cit.*, p. 385) but methods using phenylhydrazine (Ling and Nanji, *Biochem. J.*, 1921, **15**, 466), potassium hydrogen sulphite (Jolles, *Ber.*, 1906, **39**, 96) and chloramine-T (Bianda, *Ann. Chim. appl.*, 1941, **31**, 31) have also been used. Of the colorimetric methods the only important ones are those dependent upon the colour reactions given by furfuraldehyde with either aniline acetate (Stillings and Browning, *Ind. Eng. Chem. Anal.*, 1940, **12**, 499; Duncan, *ibid.*, 1943, **15**, 162; Reaves and Munro, *ibid.*, 1940, **12**, 551; Bryant, Palmer, and Joseph, *ibid.*, 1944, **16**, 74) or with Bial's orcinol reagent (McRary and Slattery, *Arch. Biochem.*, 1945, **6**, 151).

Of these three types of method only the colorimetric ones are micro-methods in the sense that they can be used for the determination of so little as one milligram of pentose, and in fact the aniline acetate method has been so used by other workers. It has been claimed that this method can also be used to differentiate between furfuraldehyde, methylfurfuraldehyde, and hydroxymethylfurfuraldehyde by using suitable colour filters (Stillings and Browning, *loc. cit.*).

The present communication describes a method of determination of pentoses by dehydration with phosphoric acid followed by distillation. The furfuraldehyde so obtained is determined by means of its intense absorption band with a maximum at 2785 A. It has been found that the intensity of light absorption of the furfuraldehyde solutions obtained from pentoses

gives quantitatively reproducible results and that there is a linear relationship between the observed light absorption of the furfuraldehyde and the amount of pentose originally present. Further, this spectroscopic method can be carried out much more rapidly than any of the other methods even when the absorption spectra are determined photographically, whilst, with a photoelectric spectrophotometer, the results can be obtained within ten minutes of making the furfuraldehyde solution.

In its present form, the method is only suitable for the determination of pentoses after separation from other sugars, but we are now investigating the possibility of using it for the determination of pentoses in mixtures of sugars.

The absorption spectrum of furfuraldehyde in hexane exhibits two main maxima near 2700 A. (log ϵ = 3·8) and 3300 A. (log ϵ = 1·5) according to Menczal (*Z. physikal. Chem.*, 1927, **125**, 161) but the more intense band is displaced to 2785 A. in aqueous solution. On account of the difficulty of obtaining and keeping really pure furfuraldehyde because of the ease with which it oxidises or polymerises, or both, it is extremely difficult to obtain consistent and reproducible values for the intensity of absorption. It was our original intention to determine this value and to use it to determine the amount of furfuraldehyde and hence of pentose in our solution. In view of the very variable stability of the rich furfuraldehyde preparations

FIG. 1.

I=D-Xylose. II=D-Ribose. III=L-Arabinose.
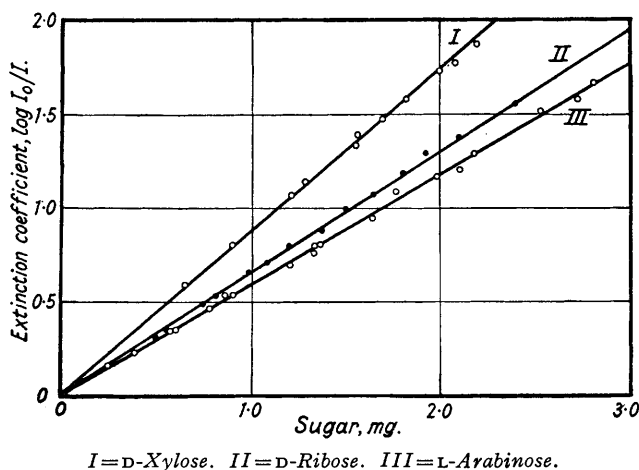
it was decided to standardise the method by plotting the observed intensities of absorption of the furfuraldehyde against the initial weights of pure pentose taken. This was found to give reproducible results simply because the dilute aqueous solutions of furfuraldehyde are unexpectedly much more stable than concentrated preparations. Furthermore, this method is independent of the absolute value of the intensity of absorption of pure furfuraldehyde which is somewhat uncertain but, according to our own observations, the molecular extinction coefficient ϵ, at the maximum (2785 A.) is of the order of 18,000 in aqueous solution.

Precise details of the method which was finally developed are given in the Experimental section of this paper, the results obtained with the pure pentoses, arabinose, xylose, and ribose (a pure specimen of lyxose to complete the series was not available at the time that this work was carried out) being shown in Fig. 1. Here the optical density $E = \log I_0/I$, which is obtained by direct reading from the photoelectric spectrophotometer, is plotted against the weight in mg. of each pentose to give a graph that can be used to determine the amount of the particular pentose in any sample.

The results show that arabinose, ribose, and xylose can be determined in amounts between 0·5 and 2·0 mg. with an error of less than ±2%. Smaller quantities can be determined easily but the percentage error is greater. The relation between intensity of absorption of furfuraldehyde and weight of pentose can be expressed as E/w, where $E = \log I_0/I$ at 2785 A. for a 2-cm. cell of the aqueous furfuraldehyde distillate made up to a known volume, *e.g.*, 100 ml., with water, and w equals the number of mg. of pentose taken. The data obtained on the three pure pentoses are shown in Table I together with criteria of purity.

TABLE I.

Pentose.	M. p.	$[\alpha]_D^{18°}$ (in H_2O).	Mean E/w.	Mean deviation.	Maximum deviation.
D-Xylose	148—149°	+ 18·9° (2·4%)	0·874	±1·53%	2·98%
D-Ribose	93·5—95	− 19·7 (1·98)	0·650	±1·24	3·84
L-Arabinose	158—160	+105·2 (1·19)	0·590	±1·83	6·78

It has been known for a long time that the dehydration of pentoses into furfuraldehyde is by no means quantitative and in fact varies from sugar to sugar. Fortunately, the percentage conversion of any one pentose is sufficiently reproducible to form the basis of various methods of determination. This reproducibility has already been demonstrated (Fig. 1) whilst the difference in the furfuraldehyde yield from different pentoses is shown in Table I by the difference in the values of E/w. These values would be identical for all three sugars if the furfuraldehyde yield were the same in each case, but the results show that xylose gives a higher yield of furfuraldehyde than does either ribose or arabinose.

After preparing the standard curves for each of the three pentoses, using a sufficient number of determinations to enable a good mean line to be drawn, a further set of determinations was carried out on samples of xylose and arabinose obtained from independent sources. The results are shown in Table II.

TABLE II.

D-Xylose, m. p. 148—149°, $[\alpha]_D^{18°}$ + 18·7°.

Wt. (mg.) taken	0·900	1·200	1·725	2·013	2·300	0·575
Wt. (mg.), calc.	0·907	1·196	1·702	2·007	2·281	0·563
Error, %	+0·777	−0·333	−1·333	−0·298	−0·739	−2·085

L-Arabinose, m. p. 158—160°, $[\alpha]_D^{18°}$ + 105·5°.

Wt. (mg.) taken	0·266	0·531	0·797	1·062
Wt. (mg.), calc.	0·263	0·528	0·809	1·048
Error, %	−1·128	−0·566	+1·510	−1·318

D-Glucose, m. p. 145—147°, $[\alpha]_D^{18°}$ + 50·7°.

Wt. (mg.) taken	2·540	3·810	7·620
Wt. (mg.), calc.	2·695	3·725	7·260
Error, %	+6·10	−2·23	−4·72

Ribose was not examined in this way as a second sample from an independent source was not available.

It is an experimental fact of long standing that the heating of acid solutions of hexoses produces extensive brown colouration, the nature of which has not yet been ascertained (cf. Singh, Dean, and Cantor, *J. Amer. Chem. Soc.*, 1948, **70**, 517). It is also known that the decomposition of hexoses to 5-hydroxymethylfurfuraldehyde takes place through other intermediate compounds and further that this compound is more labile than is furfuraldehyde (Wolfrom, Schuetz, and Cavalieri, *J. Amer. Chem. Soc.*, 1948, **70**, 514; Pascu and Miller, *ibid.*, p. 523). It is not surprising to find, therefore, that glucose and galactose, for example, give very low yields of 5-hydroxymethylfurfuraldehyde of the order of 12% or less or that the figures are not reproducible to anything like the same degree as for xylose which gives an 88% yield of furfuraldehyde. This is especially noticeable with galactose where deviations in results up to 17% were obtained and furthermore two different samples gave different yields of 5-hydroxymethylfurfuraldehyde. During the distillations with acid, glucose and galactose formed deep brown colours whereas rhamnose and galacturonic acid turned only yellow and the pentoses remained colourless under the same conditions.

The absorption spectrum of hydroxymethylfurfuraldehyde has its maximum situated at 2785 A. exactly as in the case of furfuraldehyde, but methylfurfuraldehyde (obtained from rhamnose) has ϵ_{max} at 2920 A. so that readings on this methylpentose must be taken at this higher wave-length.

The results of the spectrophotometric evaluation of the substituted furfuraldehydes obtained from various sugars other than simple pentoses are shown in Fig. 2. It will be seen that the light intensity measured at the absorption maximum in each case is, within narrow limits of error, directly proportional to the amount of sugar originally present and the mean curves shown can be used to determine small amounts of these sugar derivatives just as in the case of the pentoses already discussed.

The more nearly quantitative is the decomposition of the sugar the higher will be the E/w ratio (apart from differences in molar weight). The wide difference in various sugars, especially when we extend our study to the hexoses where the yield of furfuraldehyde derivatives is known to fall short of the maximum possible, is shown in Fig. 3. Here, the steeper the slope of the line the more nearly maximal is the production of furfuraldehyde and xylose is seen to give the highest yield of furfuraldehyde per gram whilst glucose, yielding hydroxymethyl-furfuraldehyde, gives the lowest yield of this series. Galactose was in fact studied but the yield of furfuraldehyde is so low and so poorly reproducible as to make any quantitative method, dependent upon it, quite useless.
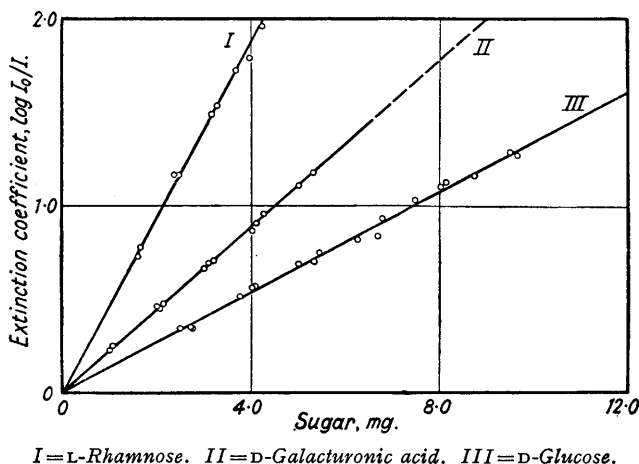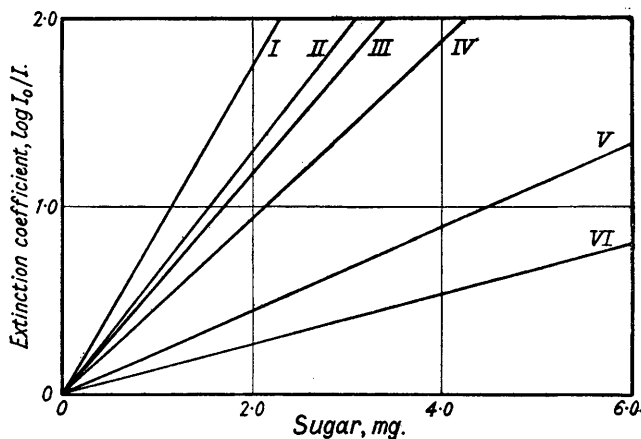
FIG. 2.

$I=$L-Rhamnose. $II=$D-Galacturonic acid. $III=$D-Glucose.

FIG. 3.

$I=$D-Xylose. $II=$D-Ribose. $III=$L-Arabinose. $IV=$L-Rhamnose.
$V=$D-Galacturonic acid. $VI=$D-Glucose.

TABLE III.

Sugar.	M. p.	$[\alpha]_D^{18°}$ (in H_2O).	Mean E/w.	Mean deviation.	Maximum deviation.
D-Galacturonic acid	—	$+55\cdot9°$ ($0\cdot411\%$)	$0\cdot222$	$\pm1\cdot14\%$	$4\cdot77\%$
L-Rhamnose (a)	93—94°	$+ 9\cdot2$ ($1\cdot95$)	$0\cdot461$	$\pm2\cdot33$	$10\cdot33$
(b)	92—94	$+ 9\cdot15$ ($3\cdot28$)	$0\cdot471$	$\pm1\cdot56$	$4\cdot03$
D-Glucose	145—147	$+52\cdot8$ ($1\cdot04$)	$0\cdot134$	$\pm2\cdot73$	$7\cdot0$

<div align="center">EXPERIMENTAL.</div>

Distillation Apparatus.—The apparatus that we have found most useful is one made wholly of glass with " Quickfit " joints, the temperature of the acid mixture being controlled by means of a short-range Anschütz thermometer (140—190°) attached to the steam-inlet tube by means of platinum wire (see diagram).

0 4 8 12 16 cm.
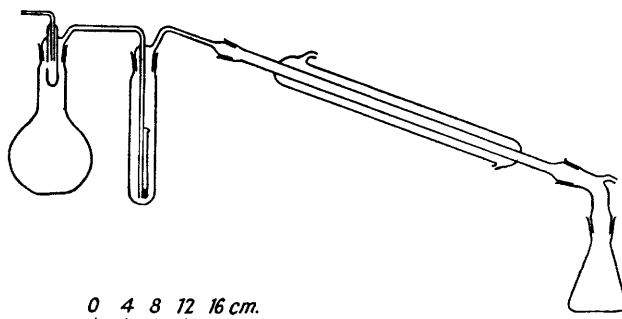
Distillation Procedure.—The method employed was based on that used by Bryant, Palmer, and Joseph (*loc. cit.*). A known volume (0·5—1·5 c.c.) of sugar solution (0·1 to 0·5% according to the sugar) was transferred by means of a graduated pipette into the distillation tube, followed by phosphoric acid (5 c.c. of 85% syrupy H_3PO_4, *d* 1·689). The tube was connected to the apparatus, steam passed through and the reaction vessel heated with a microburner to bring the temperature as rapidly as possible to 170°. The temperature was maintained at 165—175° throughout the distillation. When the whole of the furfuraldehyde had distilled (tested with aniline acetate paper) the distillate was made up to known volume (100 c.c. or 250 c.c.) with water. The intensity of absorption at 2785 or 2920 A. was then determined (1 cm. cell) and the optical density ($E = \log I_0/I$) plotted against the weight of sugar in mg. (w).

Absorption Spectra.—Determinations were made on a Beckmann DU spectrophotometer, the wavelength scale being set at 2785 A. for furfuraldehyde and hydroxymethylfurfuraldehyde, and at 2920 A. for methylfurfuraldehyde (*ex* rhamnose). Water was used as solvent in all cases and 1 cm. silica absorption cells were used under standard conditions.

Results.—The value of E/w for a given sugar is approximately constant. The slope of the graph is a measure of the yield of furfuraldehyde or its analogue from the particular sugar. If we assume that xylose gives an 88% yield [as calculated from Krober's tables (cf. Dorée, *op. cit.*, p. 377)] then the E/w values obtained here indicate that ribose and arabinose yield about 65 and 59% of the maximum possible, respectively. Galacturonic acid gives about 22% of the possible yield of furfuraldehyde.

The sugar samples examined were pure specimens obtained commercially and recrystallised until the physical constants showed no change. Wherever possible a second specimen was obtained from an independent source and the results confirmed (cf. Tables I, II, and III).