The Reaction of Pentoses with Anthrone

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Since Dreywood's (1946) original report the reaction of hexoses with anthrone in strong sulphuric acid has been widely used as a method for determining trace amounts of these sugars and their polymers (e.g. Trevelyan & Harrison, 1952; Roe, 1954, 1955; Carroll, Langley & Roe, 1956). According to Sattler & Zerban (1948) pentoses and methyl pentoses should react with anthrone in a similar manner. Shriver, Webb & Swanson (1950) and Kohler (1952, 1954) reported that whereas the reaction was suitable for determining the methyl pentoses L-rhamnose and L-fucose, the colours produced by pentose sugars were very unstable to heat. Bridges (1952) attempted to overcome the instability of the pentose–anthrone complex by using a very short heating time (10 sec.). He developed a method for determining pentoses with anthrone, but colour fading is likely to occur unless the prescribed conditions are rigidly followed.

As a result of these reports it has been assumed that the anthrone reaction is not particularly suitable for measuring pentoses (e.g. see Ashwell, 1957). Unless they are present in very high concentration it has also been implied that pentoses will not interfere in hexose determinations.

In all of the work reported on pentoses, and generally with hexoses, from 5 to 10 mg. of anthrone was mixed with 20–150 μg. of sugar. The final acid concentration in the reaction was between 60 and 66 % (v/v). Jermyn (1955) reported satisfactory results in measuring hexoses with a reagent containing only 0·02 % (w/v) of anthrone in 70 % (v/v) sulphuric acid. In this case only 2 mg. of anthrone was mixed with 20–100 μg. of sugar in a final acid strength of 63 % (v/v). Jermyn (1956) also reported that at room temperature this reagent slowly produced colour with pentoses and rhamnose. Much more colour was produced under these conditions by hexuloses, but practically none by aldohexoses. With Jermyn's (1955) experimental conditions we found that pentoses reacted in a manner which was quite different from that described by Kohler (1952). Strong greenish-blue colours were produced which appeared to be as stable to heat as those produced by D-glucose and L-rhamnose.

Although several methods are available for determining pentose sugars, it was thought that an adaptation of the relatively simple anthrone method for pentose estimations would be of value. The reaction of pentoses with anthrone has therefore been reinvestigated. The present paper reports the results obtained with D-xylose, D- and L-arabinose and D-ribose. Results for D-glucose and the methyl pentose L-rhamnose are included for comparative purposes. As a result of this work a rapid and easy method for measuring pentose sugars with anthrone has been developed and is described.

EXPERIMENTAL

Sugars. The sugars used were D-xylose, D- and L-arabinose, D-ribose, L-rhamnose and D-glucose. All of them were shown to be chromatographically pure by the usual solvent techniques and sprays (Partridge, 1949; Trevelyan, Procter & Harrison, 1950). After vacuum drying of each sugar at 61° a standard solution was prepared to contain 100 μg. of sugar/ml. of soln.

Anthrone reagent. Anthrone (5–50 mg.) was dissolved in H_2SO_4 which was prepared by adding concentrated acid (70 ml.) to water (30 ml.). All anthrone solutions were aged for at least 2 hr. and were discarded after 24 hr. They were kept at 0°. The slight variations between the different sets of results reported are due to the instability of anthrone in H_2SO_4 and the resulting variation in the composition of the reagent. Such variations are normally allowed for in hexose determinations by including a standard sugar solution in each batch of analyses.

Reaction of anthrone and sugar. Portions of the sugar solution (0·2–1 ml.) were pipetted into large boiling tubes (20 cm. × 25 mm.) and diluted to 1 ml. with water where necessary. Each tube was placed in a bath of cold water and agitated whilst anthrone reagent (10 ml.) was slowly added. The tubes were stoppered and, after their contents were thoroughly mixed, transferred to a vigorously boiling water bath for the specified time. They were then placed in a cold-water bath and stored in the dark for 30 min. before the colour produced was measured. A blank consisting of water (1 ml.) and anthrone reagent (10 ml.) was included in each batch of analyses in order to eliminate colour effects due to the anthrone.

The optical densities of the coloured solutions were read on a Beckman model DU spectrophotometer with cells of 1 cm. thickness. The bluish-green solutions produced by hexoses and the greener solutions of the pentoses have an absorption maximum at 625 mμ, and this wavelength was therefore used. The usual precautions were taken throughout the analyses to keep the reacting solutions dust-free.

RESULTS

Effect of anthrone concentration on the reaction. Samples (1 ml.) of each sugar solution were treated with a series of anthrone reagents containing 0·005–0·05% (w/v) of anthrone in the 70% (v/v) sulphuric acid. Preliminary work with 0·02% (w/v) of anthrone had shown that good colour was obtained from pentoses after heating for 7 min. This time of heating was therefore used. Jermyn (1955) used a heating time of 7·5 min. when determining hexoses under similar conditions.

The results obtained for each sugar are shown in Fig. 1 as a plot of optical density, at 625 mμ, against the anthrone concentration of the acid reagent. The curves obtained for D-xylose, L-arabinose and D-ribose are all of the same type, with a maximum at about 0·01% (w/v) of anthrone. They are strikingly different, however, from the curves obtained for D-glucose and L-rhamnose. It appears that excess of anthrone rapidly destroys the greenish-blue colour produced by the pentoses. Maximum colour production is obtained from pentoses when only 1 mg. of anthrone reacts with 20–100 μg. of sugar. Previous workers used from five to ten times as much anthrone.

In all subsequent work with pentoses an anthrone concentration of 0·01% (w/v) in the acid reagent was used. With D-glucose and L-rhamnose optimum results are obtained with 0·04–0·05% (w/v) of anthrone. An anthrone concentration of 0·02% (w/v) was used, however, as at this level the colour produced was within the range of colour intensity given by the pentoses and provided a suitable comparison. It can be seen from Fig. 1 that anthrone concentration is not nearly so critical in determining hexoses as it is with pentoses.

Effect of acid concentration on the reaction. Anthrone reagents containing 0·01% (w/v) of anthrone in sulphuric acid, which ranged from 64 to 75% (v/v), were allowed to react with the pentoses (1 ml.) under standard conditions. Maximum absorption at 625 mμ was obtained with 70% (v/v) acid. Typical extinction values for D-xylose were: with 65% acid, 0·30; with 70% acid, 0·48; with 75% acid, 0·37. The weaker acid appeared to produce less of the blue–green colour and the stronger acid (75%) produced a distinctly yellower colour. The acid strength of the reagent was therefore standardized at 70% (v/v), giving a reaction mixture which was 63% (v/v).

Effect of heating time on the colour complex. Experiments were carried out with each sugar (100 μg.) and its appropriate anthrone reagent for different lengths of time in the boiling-water bath. The results obtained with D-xylose, D-ribose and D-glucose are shown in Fig. 2 as a plot of optical density against time at 100°. L-Arabinose gave a curve which was almost identical with that obtained with D-glucose, and L-rhamnose with that for D-xylose. With all sugars maximum colour formation was obtained within 6–8 min. heating, followed by a very gradual fading. A heating time

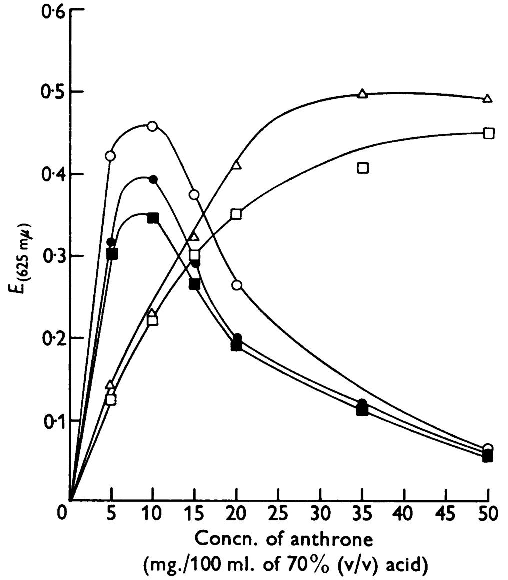

Fig. 1. Effect of anthrone concentration on the optical density of the coloured solution produced from 100 μg. of sugar after 7 min. at 100°. ○, D-Xylose; ■, L-arabinose; ●, D-ribose; △, L-rhamnose; □, D-glucose.

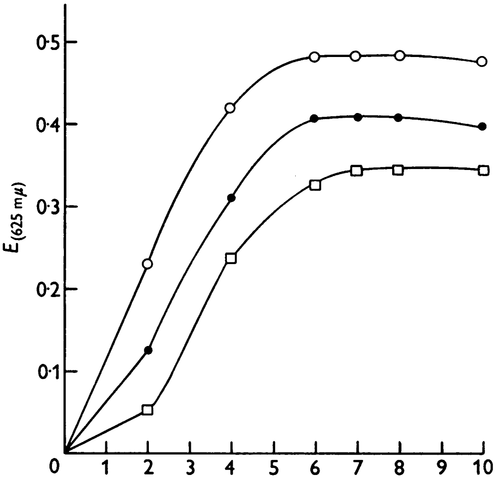

Fig. 2. Effect of heating time on the optical density of the coloured solution produced from 100 μg. of sugar. D-Xylose (○) and D-ribose (●) with 0·01% (w/v) of anthrone reagent, and D-glucose (□) with 0·02% (w/v) of anthrone.

of 7 min. is thus satisfactory for determining pentoses as well as hexoses, provided that the correct anthrone concentration is used.

Absorption spectra of the developed colours. The absorption spectra for the coloured solutions produced by D-xylose, L-rhamnose and D-glucose over the wavelength range 400–680 mμ, and with the optimum conditions defined above, are shown in Fig. 3. L-Arabinose and D-ribose gave spectra which were identical with that of D-xylose. In each there is an absorption maximum at 625 mμ and this wavelength may be used for all analyses. The change from the clear-blue colour produced by L-rhamnose to the greener colours formed by pentoses is related to the second absorption maximum in the 420–450 mμ region.

Standard method for determining pentoses with anthrone. The standard conditions for determining pentose sugars may now be defined as follows. Anthrone reagent (10 ml.) containing 0·01 % (w/v) of anthrone in 70 % (v/v) sulphuric acid is added, with cooling, to the sugar solution (1 ml.), which contains 20–100 μg. of sugar. After heating for 7 min. at 100° and cooling for 30 min. the extinction at 625 mμ is measured. Concentration curves obtained with D-xylose, L-arabinose and D-ribose are given in Fig. 4 (*a*). Results with D-arabinose were identical with those for L-arabinose. For comparison the concentration curves obtained with D-glucose and L-rhamnose are shown in Fig. 4 (*b*). For these two last-named sugars the anthrone concentration was increased to 0·02 % (w/v) in the acid and the other conditions remained the same. It can be seen that the colours produced are proportional to the concentration of pentose and may be used for estimating these sugars, at least in the range 20–100 μg./ml.

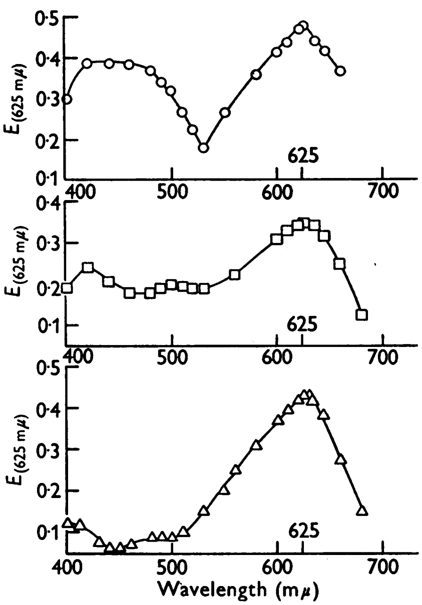

Fig. 3. Absorption spectra of the coloured solutions produced by 100 μg. of sugar after heating with anthrone for 7 min. at 100°. D-Xylose (○) with 0·01 % (w/v) of anthrone reagent; D-glucose (□) and L-rhamnose (△) with 0·02 % (w/v) of reagent.

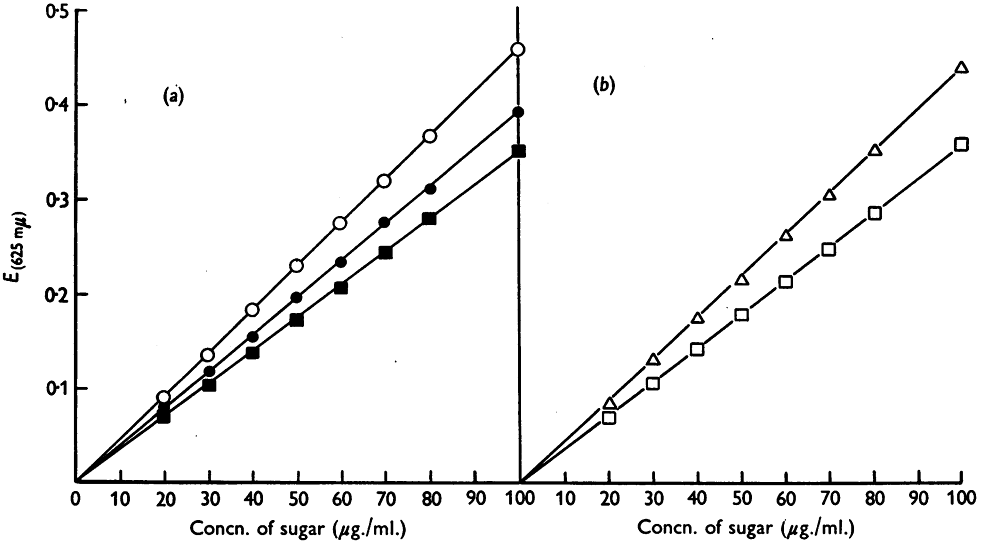

Fig. 4. Relationship of sugar concentration (μg./ml.) to optical density after reaction with anthrone reagent for 7 min. at 100°. (*a*) ○, D-Xylose; ●, D-ribose; ■, L-arabinose. Anthrone, 0·01 % in 70 % (v/v) acid. (*b*) □, D-Glucose and △, L-rhamnose. Anthrone, 0·02 % in 70 % (v/v) acid.

Reaction of pentose polymers with anthrone. Hexose polymers are readily hydrolysed in the strong acid and then react with anthrone to give the normal colour (Morris, 1948; Roe, 1954). Pentose polymers might be expected to do the same. Unfortunately samples of xylose and arabinose polymers were not available for testing. The only ribose polymer available to us was a sample of yeast nucleic acid (British Drug Houses Ltd.; 'laboratory grade'). With the standard conditions for pentoses this material (100 μg.) gave a greenish-blue colour equivalent to 27 % of D-ribose (calculated for pure yeast nucleic acid, 46 % of ribose).

DISCUSSION

According to Sattler & Zerban (1948) the anthrone reaction is dependent on the conversion of the sugars by acid into furfuraldehyde derivatives, which then react with the anthrone. Shriver *et al.* (1950) demonstrated that such a mechanism was in fact involved. In this case D-glucose would produce hydroxymethylfurfuraldehyde, L-rhamnose methylfurfuraldehyde and the pentoses furfuraldehyde. The present work suggests that the reported instability of the pentose colour is due mainly to the presence of excess of anthrone. It is possible that there is a reaction between the furfuraldehyde–anthrone complex which is formed and excess of anthrone, to produce a yellow or colourless compound. The methyl group present in the furfuraldehyde derivatives of hexoses and methyl pentoses may prevent or considerably slow down such a reaction.

The postulated decoloration of the coloured complex seems to be dependent on the absolute concentration of the excess of anthrone rather than on the relative amounts of anthrone and pentose initially present. Fig. 4 (*a*) shows that with decreasing concentrations of pentose, and hence increasing relative amounts of anthrone, colour production remains proportional to sugar concentration, so presumably no colour destruction is occurring. A more detailed investigation of the mechanism of the reaction between pentoses and anthrone would possibly explain this effect of concentration.

Provided that the correct anthrone concentration in the final reaction mixture is used, pentoses may be easily determined by the anthrone reaction. As the colour is relatively stable to heat, no special precautions, other than those used for hexoses, are necessary. Under the conditions used by most authors for determining hexoses, pentoses will not normally produce much colour, although methyl pentoses will do so. With Jermyn's (1955) experimental conditions, however, considerable interference from pentoses will be encountered.

SUMMARY

1. The reaction of the pentoses D-xylose, D- and L-arabinose and D-ribose with anthrone has been investigated.

2. Production of a heat-stable blue–green colour by these sugars has been shown to be primarily dependent on the concentration of anthrone. Excess of anthrone appears to destroy the colour rapidly.

3. The conditions for determining pentose sugars by the anthrone reaction have been defined.

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