192. *The Quantitative Estimation of Xylose.*

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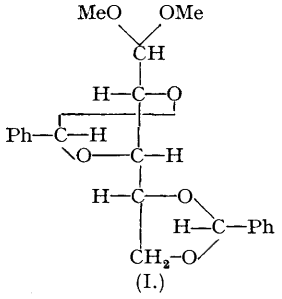
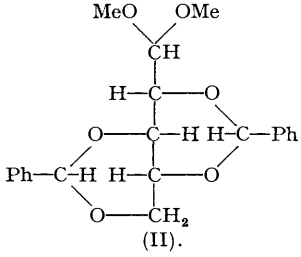
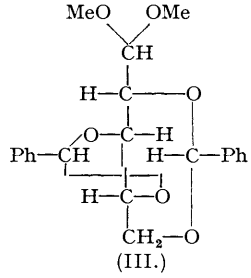
Xylose condenses with benzaldehyde dissolved in methyl alcoholic hydrogen chloride forming the sparingly soluble *dibenzylidene dimethyl acetal* of *xylose*. A quantitative method of estimation of the sugar has been developed based on the formation of this derivative.

The quantitative estimation of xylose in the presence of other sugars is a matter of considerable difficulty, which is augmented when other pentoses or methyl pentoses are present [compare Wise and Appling, *Ind. Eng. Chem.* (*Anal. Ed.*), 1945, **17**, 182]. The estimation of arabinose alone is a comparatively simple matter, but when arabinose and xylose occur together, as in damson gum (Hirst and Jones, *J.*, 1938, 1174), no satisfactory method for the direct estimation of xylose has hitherto been available. The usual method of detecting xylose is to oxidise the mixture of sugars with bromine in the presence of cadmium carbonate. On evaporation of the filtered solution boat-shaped crystals of the double salt, cadmium bromide–cadmium xylonate, separate if xylose is present. This method is not quantitative, and becomes uncertain when only small quantities of sugar are available. To get over this difficulty, attempts were made to find a reagent which was specific for xylose. It was then found that benzaldehyde dissolved in methanolic hydrogen chloride reacted with xylose forming a sparingly soluble crystalline derivative. Under similar conditions no such crystalline derivative was obtained from glucose, mannose, galactose, fructose, sorbose, maltose, sucrose, α-methylmannoside, α-methylglucoside, rhamnose and arabinose. Analysis of the product from xylose showed it to be the *dimethyl acetal* of *dibenzylidene* d-*xylose*, for which three structural formulæ are possible (I), (II), and (III). Since


(I.)


(II).


(III.)

methylation with Purdie's reagents did not increase the methoxyl content, it would seem that the substance has no free hydroxyl groups.

Because of the low solubility of this derivative of xylose, it was possible to detect traces of xylose and to develop a quantitative method of estimation of the sugar accurate to within 8% (calculated on the weight of xylose used) when estimating between 0·2 and 0·8 g. of xylose. To standardise the method, various quantities of xylose were added to 10 c.c. of the reagent and after seven days the weight of derivative was determined. The weights of derivative and of xylose were then plotted and the points found to lie approximately on a straight line the equation of which was $y = 0{\cdot}482x + 0{\cdot}055$, where y = the weight of xylose and x = the weight of derivative isolated under standard conditions. From this equation and from the weight of derivative isolated it is possible to estimate the amount of xylose present.

Experimental.

Reagent. The reagent was prepared by dissolving redistilled benzaldehyde (40 c.c.) in a mixture of 2·5N (by volume) methanolic hydrogen chloride (20 c.c.) and methanol (120 c.c.). Xylose was weighed out and added to the reagent (10 c.c.) in a stoppered flask and the mixture left for seven days at room temperature with occasional shaking. The crystalline derivative was then filtered off, washed successively with water (200 c.c.) and methanol (40 c.c.) then dried at 100° for 1 hour and weighed. The weights of derivative isolated from various weights of xylose are given in Table I.

Table I.

Xylose (g.)	0·068	0·162	0·287	0·474	0·789	1·145
Dibenzylidene dimethyl acetal of xylose (g.)	0·010	0·134	0·455	0·896	1·542	2·240

The *dimethyl acetal* of *dibenzylidene* d-*xylose* crystallised in needles. It was very sparingly soluble in all the usual solvents, but was slightly more soluble in chloroform. It was unaffected by Purdie's reagents, showing the absence of free hydroxyl groups and on steam distillation in the presence of dilute sulphuric acid it underwent hydrolysis with the formation of steam-volatile benzaldehyde and methanol, whilst xylose remained in solution thus providing a convenient method for isolating xylose from mixtures of sugars. The derivative had m. p. 211° after recrystallisation from chloroform–ligroin (40—60°) and analysed as the dimethyl acetal of dibenzylidene *d*-xylose. $[\alpha]_D^{20°}$ −9° (*c*, 1·2 in chloroform) (Found : C, 67·7; H, 6·3; OMe, 17·1%. $C_{21}H_{24}O_6$ requires C, 67·7; H, 6·5; OMe, 16·7%).

Since the usual sugars associated with xylose in the plant gums are arabinose and galactose, artificial mixtures of these sugars with xylose were made and analysed for xylose by the method given above. The results of these analyses are given in Table II, in which Column 5 gives the weight of xylose estimated and Column 1 the weight of xylose present in the mixture. It is seen that the method gives results accurate to within some 8%, even under unfavourable conditions. This represents a very considerable improvement on other methods for the estimation of xylose in mixtures.

Table II.

Xylose, g.	Arabinose, g.	Galactose, g.	Derivative of xylose, g.	Xylose (from equation), g.	Error in g.	% error calculated on the wt. of xylose weighed out.
0·467	—	—	0·768	0·430	−0·037	−7·9
0·277	—	—	0·442	0·272	−0·005	−1·8
0·432	1·000	—	0·700	0·398	−0·034	−7·9
0·502	—	1·05	0·860	0·475	−0·027	−5·4
0·406	—	1·130	0·660	0·380	−0·026	−6·4
0·410	0·590	0·654	0·725	0·410	—	—
0·348	0·330	—	0·585	0·342	−0·006	−1·7
0·499	—	0·361	0·901	0·496	−0·003	−0·6
0·717	0·138	—	1·253	0·660	−0·057	−8·1
0·339	—	0·534	0·569	0·332	−0·007	−2·1

In carrying out the estimation, syrups should not weigh more than 1·5 g. and should be freed from all traces of moisture. With smaller quantities of xylose (25 mg. to 150 mg.) it is advisable to use 1 c.c. of the reagent. The reagent can be used as a spot test for xylose since a crystalline precipitate forms with 10 mg. of dry sugar and a spot or two of reagent.

The authors wish to thank Professor E. L. Hirst, F.R.S., for his interest and advice.

The University, Bristol. [*Received, May 11th*, 1945.]