A Method for the Determination of Reducing Disaccharides in the Presence of Aldohexoses

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This method is suitable for the study of glucosidases and especially those, *e.g.* isomaltases, which are difficult to determine [1] with the Tauber and Kleiner hexose sensitive reagent [2], and where a method is wanted that requires very little material. It seems possible also to use the method for determining higher oligosaccharides in mixtures with aldohexoses.

The principle of the method is an oxidation of the sugar mixture to the corresponding aldonic and aldobionic acids. The aldonic acid does not give any colour reaction when heated with anthrone in sulfuric acid, whereas the aldobionic acid — due to the non oxidized glucose residue that is liberated by the strong acid solution — will react with the anthrone reagent giving a blue-green colour reaction. The oxidation of the sugar mixture is performed at room temperature with an alkaline hypobromite solution. Keeping the hypobromite solution not too alkaline the over-oxidation of the aldobionic acid is negligible. After the oxidation excess hypobromite is destroyed with thiourea, and the determination proceeds using one of the anthrone methods.

Alkaline hypobromite reagent. 2.5 ml bromine is dissolved in 250 ml ice-cold 1 N NaOH and diluted to 400 ml with water. Kept in the refrigerator in a brown bottle this reagent is stable at least for 2 months. From this stock solution a hypobromite solution is prepared immediately before use by adding 5 ml 1.5 N H_2SO_4 to 20 ml of the stock solution.

Thiourea reagent. 7.5 g thiourea in 100 ml 0.5 N H_2SO_4.

Anthrone reagent. 0.2 g anthrone in 100 ml 95 % H_2SO_4 (prepared daily).

Procedure. Pipet 1 ml of each sample, having a disaccharide content $\leq 180 \times 10^{-3}$ mg, into a test tube. Include two blanks and two standards (see below). Add 0.5 ml of the diluted hypobromite reagent and mix carefully. The hypobromite reagent is added to each tube with intervals of, *e.g.*, 30 sec. After reaction for 20 min add 0.5 ml of the thiourea reagent and mix carefully after each of the additions, the thiourea beeing added with the same intervals as the hypobromite (constriction pipets are used). The analysis proceeds now using one of the modifications of the anthrone method, *e.g.* the conditions used by McCready *et al.*[3]: 4 ml of the anthrone reagent is added to the samples while cooling in a water bath. Mix thoroughly while cooling and heat the samples for 7.5 min at 100°C. Cool the tubes rapidly to 25° C and measure in a colorimeter at 625 mμ (or use a corresponding filter) in a 1 cm cell.

In each set of samples are included two blanks and two standards, the blanks containing 10^{-6} mole monosaccharide and the standards 0.5×10^{-6} mole disaccharide. Calculation of disaccharide (mole):

Table 1. Determination of some disaccharides in the presence of their corresponding monosaccharides.

Disaccharide mole × 10^6	Monosaccharide mole × 10^6	Disaccharide found mole × 10^6
Maltose	Glucose	Maltose
0.050	0.900	0.051
0.150	0.700	0.151
0.250	0.500	0.253
0.350	0.300	0.348
0.450	0.100	0.447
Isomaltose	Glucose	Isomaltose
0.050	0.900	0.051
0.150	0.700	0.151
0.250	0.500	0.252
0.350	0.300	0.352
0.450	0.100	0.451
Cellobiose	Glucose	Cellobiose
0.100	0.800	0.100
0.200	0.600	0.201
0.300	0.400	0.303
0.400	0.200	0.401
0.500	0.000	0.500
Lactose	Glucose/Galactose	Lactose
0.100	0.800	0.097
0.200	0.600	0.196
0.300	0.400	0.303
0.400	0.200	0.401
0.500	0.000	0.495

$$\frac{\text{Reading of sample} - \text{reading of blank}}{\text{Reading of standard} - \text{reading of blank}} \times 0.5 \times 10^{-6}$$

Normally the blank with monosaccharide will give nearly the same value as a water blank, and an extraordinarily high monosaccharide blank may be due to a too old hypobromite reagent.

In Table 1 are shown some examples of determinations. The amount of monosaccharide corresponds to different degrees of hydrolysis of the disaccharide. The blank corresponds to 100 % of hydrolysis. It is also possible to have greater amounts of monosaccharide (2—3 times the amounts used in the examples). In determining, *e.g.*, lactose the blank should contain half glucose and half galactose.

Ketohexoses will not be fully oxidized under the conditions given; *e.g.* fructose will need an oxidation time of at least 1 hour to give a negative anthrone reaction. This difference in oxidation rate has been used for the determination of fructose in the presence of glucose by Slein and Schneel [4].

Small amounts of protein and acetate and citrate buffers are without influence on the determination; if necessary the pH must be adjusted to about 10 before the addition of hypobromite.

If a faster method is wanted, one of the less accurate "heat of mixing" anthrone methods may be used [5].

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Preliminary Results of an Electron Diffraction Reinvestigation of *Cyclo*butane and *Cyclo*pentane

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The molecular structure of *cyclo*butane and *cyclo*pentane have been reinvestigated by the electron diffraction sector method. However, further refinements and a more extensive analysis of the diffraction data have to be performed before the exact equilibrium conformation of the ring-formed carbon skeletons can be determined. Only some preliminary results therefore will be presented in this note.

As members of a group of molecules characterized by a strained conformation of the carbon skeleton, *cyclo*butane and *cyclo*pentane have been the object of a series of experimental and theoretical studies [1-16].

By this investigation we hope to be able to obtain results for the molecular structures which are more extensive and more accurate than those previously published. A comparison of the present results with unpublished structure data obtained by the same diffraction unit in 1956—1957, is also of great interest because it provides an opportunity for estimating the reproducibility of the results obtained by this method.

Cyclobutane. By an analysis of the intensity curve and of several radial distribution curves obtained by using different damping functions, the following values for the C—C and C—H bond distances have been obtained:

C—C: 1.548 ± 0.003 Å and C—H: 1.092 ± 0.010 Å.

The values found by the unpublished investigation in 1956—1957 are 1.547 Å and 1.089 Å, respectively.

These results demonstrate that the C—C bond distance in a saturated 4-membered ring is longer than the corresponding distance in the linear paraffins. For ethane the values 1.536 Å [17] and 1.534 Å [18] have been reported. Recent investigations of *n*-butane give the values 1.533 Å [19] and 1.539 Å [20]. Finally by an electron diffraction