An Ion-exchange Method for the Separation of Partially Methylated Sugars, and its Application in an Improved Preparation of 2-O-*Methyl*-D-*glucose.*

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Certain mixtures of carbohydrates may be resolved by preferential adsorption on the borate form of a strongly basic ion-exchange resin. Monosaccharides having positions 1, 2, and 4 unsubstituted can be separated on the preparative scale from those having substituents in any of these positions, and similarly, glycosides having a *cis*-glycol grouping can be separated from those not containing such a group. The value of the method is illustrated in a simplified preparation of 2-*O*-methyl-D-glucose.

The separation of partially methylated derivatives is a very common problem in carbohydrate chemistry, the difficulties being particularly acute when derivatives of a similar degree of substitution are involved. On the micro-scale, however, successful separations of this type have been achieved in certain cases by taking advantage of the formation of ionised complexes of the sugar derivatives with boric acid or alkali borates (*e.g.*, Foster and Stacey, *J. Appl. Chem.*, 1953, **3**, 19; Bell and Northcote, *Chem. and Ind.*, 1954, 1328; Barker and Smith, *ibid.*, p. 19) and it was possible by this method to separate mixtures which could not otherwise be resolved. In spite of the success of these analytical procedures, the preparative aspect of the problem appears to have been neglected.

The procedure described below utilises the borate form of the strongly basic ion-exchange resin Amberlite IRA-400, which forms strongly adsorbed complexes with certain partially methylated sugars while having little effect on others. This adsorption is clearly analogous to that originally noted for glycerol on similar resins (Zager and Doody, *Ind. Eng. Chem.*, 1951, **43**, 1070) and later utilised by Khym and Zill (*J. Amer. Chem. Soc.*, 1952, **74**, 2090) and Zill, Khym, and Cheniae (*ibid.*, 1953, **75**, 1339) in the chromatographic analysis of monosaccharide and disaccharide mixtures. The present method has been successfully used for separations of several mixtures of partially methylated sugars on the preparative scale by the batchwise use of the resin. The batch technique is simpler in operation than chromatographic procedures, and, by the use of more concentrated solutions, may be applied to much larger amounts of material.

Böeseken (*Adv. Carbohydrate Chem.*, 1949, **4**, 189) has emphasised that the most stable borate complexes of this type are formed in the 1 : 2-position with the furanose form of the sugar and accordingly we found this procedure of particular use in the separation of glucose derivatives substituted in the 2-*O*-position from those with a free 2-hydroxyl group. Thus, 100% adsorption of 3-*O*-methyl-D-glucose from its aqueous solution in contact with the resin was observed in 2 hr. under the experimental conditions employed, while 2-*O*-methyl- and 2 : 3-di-*O*-methyl-D-glucose were adsorbed slowly and incompletely. Further, the importance of the furanose structure is demonstrated by the rapid and complete adsorption of 6-*O*-methyl-D-glucose in comparison with the weak adsorption of 4 : 6-di-*O*-methyl-D-glucose. As expected, 2 : 3 : 6-tri-*O*-methyl-D-glucose was adsorbed to an even smaller extent. It seems possible, however, that the slow adsorption of 2-substituted sugars (where complex formation at $C_{(1)} : C_{(2)}$ is precluded) could be explained by the participation of the open-chain form of the sugar (cf. Foster, *J.*, 1953, 982).

The same resin may also be used to separate suitable mixtures of glycosides, and, except for the cases reported by Chambers, Zill, and Noggle (*J. Amer. Pharm. Assoc.*, 1952, **41**, 461) which depended on differences in the aglycone group, gives the first example of this type of separation by an ion-exchange resin. The separation depends on the preferential adsorption of a component having a *cis*-glycol grouping and is demonstrated in the case of methyl α-D-gluco- and -manno-pyranosides. The slight adsorption of the glucoside in this case is noteworthy and, in view of its ready removal from the resin by washing with

water, probably accords with Foster's conclusion (*loc. cit.*) that formation of a stable 4 : 6-complex requires the presence of the open-chain form of the sugar. A further application to the separation of a glycoside (not having a *cis*-glycol group) from the parent aldose has been employed in the case of glucosides as a routine procedure in these laboratories for some time, and is preferred to the procedure of Roseman, Abeles, and Dorfman (*Arch. Biochem. Biophys.*, 1952, **36**, 232) since degradation of the aldose by the basic form of the resin is avoided (cf. Phillips and Pollard, *Nature*, 1953, **171**, 41).

In all the observed cases, with the exception of methyl α-D-glucopyranoside, the sugars, when once adsorbed on the resin, were not readily removed by washing with water but were recovered in good yield by treating the resin with a solution of boric acid or sodium tetraborate, the former being preferred normally to avoid risk of alkaline degradation (cf., *e.g.*, Kenner and Richards, *J.*, 1954, 278). The pure compound was then readily obtained by a development of the method of Zill, Khym, and Cheniae (*loc. cit.*).

The principles implied in the above are expected to be of general application to various types of mixtures which are commonly obtained in carbohydrate chemistry, and are well illustrated in the following improved preparation of 2-*O*-methyl-D-glucose. The latter compound has previously been prepared by several methods (Weygand and Trauth, *Chem. Ber.*, 1952, **85**, 57; Bourne and Peat, *Adv. Carbohydrate Chem.*, 1950, **5**, 148), but each has involved a series of several reactions in order to block all hydroxyl groups except that on $C_{(2)}$. However, the known preferential reactivity of the 2-hydroxyl group compared with that at $C_{(3)}$ with alkaline reagents (Gaver *et al.*, U.S.P. 2,563,526, 1951; Heddle and Percival, *J.*, 1939, 249; Robertson and Griffith, *J.*, 1935, 1193), suggested that the partial methylation of methyl 4 : 6-*O*-benzylidene-α-D-glucoside should yield predominantly the 2-methyl ether. This was found to be the case and hydrolysis of the product yielded a mixture comprising mainly 2- and 3-*O*-methyl-D-glucoses with glucose, from a solution of which the last two components were readily removed by treatment with the borate resin as described above. Unfortunately, it was not possible to avoid the formation of a small amount of the 2 : 3-di-*O*-methyl derivative during the partial methylation and although pure 2-*O*-methyl-D-glucose could be obtained solely by separation with the borate resin, the yield was improved by use of cellulose column chromatography.

Experimental

Chromatographic separations, on Whatman No. 1 paper, were carried out with a solvent containing butan-1-ol–pyridine–water (6 : 4 : 3). The sprays used to reveal the sugars were: (a) silver nitrate (Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444); (b) *p*-anisidine hydrochloride (Hough, Jones, and Wadman, *J.*, 1950, 1702).

Preparation of the Borate Form of Amberlite Resin IRA-400.—A column of Amberlite resin IRA-400 (OH) (100 g.; 20—50 mesh) was washed with a solution of sodium tetraborate (0·1M; 5 l.), excess of borate removed by washing with water, and the resin collected by filtration and stored in a damp condition.

Adsorption of Sugars by Amberlite Resin IRA-400 (*borate*).—In typical experiments, the sugar (0·25 g.) was dissolved in water (28 ml.) and, if necessary, the solution was kept overnight to attain mutarotational equilibrium. The borate resin (5 g.) was added, the mixture shaken, and the adsorption of the sugar followed by measurement of optical rotation. In several cases the absence of rearrangement was demonstrated by evaporation of the equilibrium solution, the original compound being obtained in yield corresponding to the decrease in optical rotation. The behaviour of various sugars is recorded in the annexed Table.

Desorption of Sugars from Amberlite Resin IRA-400 (*borate*).—The resin carrying the adsorbed sugar was collected by filtration and washed (0·5 hr.) by shaking it with water (3 × 50 ml.). Examination of the washings showed that only in the case of methyl α-D-glucoside was the sugar completely eluted. However, prolonged shaking removed a small proportion (8% at equilibrium) of methyl α-D-mannoside. The resin was then shaken (1 hr.) with successive quantities of 0·5M-boric acid (50 ml.), until no further sugar was removed from the resin. The combined boric acid solutions were concentrated under reduced pressure and the residue was subjected to repeated distillation under reduced pressure with quantities (500 ml.) of redistilled methanol (cf. Zill, Khym, and Cheniae, *loc. cit.*). Complete removal of boric acid was demonstrated by electrical-conductivity measurements on an aqueous solution of the final residue.

The dried residue was weighed and crystallised by the addition of a few drops of absolute alcohol. In typical experiments crystalline 3-*O*-methyl-D-glucose was obtained (80%), while methyl α-D-mannoside (m. p. and mixed m. p. 192—193·5°) was recovered from the resin in 65% yield after only one washing with boric acid, both products being chromatographically pure.

Sugar	Rotational equilm. (hr.)	Adsorption (%) at equilm.
D-Glucose	2	100
D-Mannose	2	100
2-*O*-Methyl-D-glucose	>7	39
3-*O*-Methyl-D-glucose	2	100
6-*O*-Methyl-D-glucose	2	100
2 : 3-Di-*O*-methyl-D-glucose	>7	56
4 : 6-Di-*O*-methyl-D-glucose	>7	38
2 : 3 : 6-Tri-*O*-methyl-D-glucose	>7	8·5
Methyl α-D-glucopyranoside	3	33
Methyl α-D-mannopyranoside	3	95

Typical Separations using Amberlite Resin IRA-400 (*borate*).—(a) D-*Glucose and methyl* α-D-*glucopyranoside*. A mixture of 0·125 g. of each compound in water (28 ml.) was shaken for 4 hr. with Amberlite resin IRA-400 (borate) (5 g.), and the resin collected by filtration and washed with water (3 × 50 ml.; 2 hr. each). The filtrate and washings, on evaporation to dryness, afforded methyl α-D-glucoside (0·098 g., 78%) which crystallised completely, had m. p. and mixed m. p. 167·5—168·5°, and moved as a single component on the paper chromatogram. Chromatographically pure D-glucose (0·088 g., 70%) was obtained from the resin by elution with boric acid (4 × 50 ml.; 0·5M), as described above.

(b) *Methyl* α-D-*glucopyranoside and methyl* α-D-*mannoside*. A solution of 0·125 g. of each compound in water (28 ml.) was shaken overnight with Amberlite resin IRA-400 (borate) (5 g.). Methyl α-D-glucoside (0·087 g., 70%), obtained as described under (a), had m. p. 158—161° and was found (paper chromatography) to contain mannoside. A further brief treatment with the borate resin (1 g.) gave glucoside of m. p. and mixed m. p. 167·5—169° which contained a trace of mannoside. After removal of the last traces of glucoside with water, the resin yielded chromatographically pure methyl α-D-mannoside (0·070 g., 56%) which had m. p. and mixed m. p. 192—194°.

Preparation of 2-O-*Methyl*-β-D-*glucose*.—A solution of methyl 4 : 6-*O*-benzylidene-α-D-glucoside (20·0 g.) with sodium hydroxide (3·12 g.) in acetone (200 ml.) and water (40 ml.) was stirred and maintained at 45° while methyl sulphate (6·9 ml.) was added slowly during 1 hr. Thereafter the mixture was boiled under reflux for 30 min. (⟶ pH9), then poured into water (1 l.), and the resulting solution concentrated at 35° under reduced pressure until white needles of methyl 4 : 6-*O*-benzylidene-2 : 3-di-*O*-methyl-α-D-glucoside began to separate (2·80 g.; m. p. and mixed m. p. 122—123° when recrystallised from water). After filtration the solution was adjusted to 1·0N with concentrated sulphuric acid, heated on the boiling-water bath for 20 hr., and concentrated under reduced pressure to 200 ml. An aqueous solution of barium hydroxide (95% of the calculated amount) was added to the hot solution, and neutralisation completed by addition of barium carbonate. Examination of the resulting solution by paper chromatography (sprays " a," " b ") showed the presence of glucose, 2-*O*-methyl-, 3-*O*-methyl-, and 2 : 3-di-*O*-methyl-D-glucose, in the proportion, estimated visually, of 6 : 4 : 1 : 1. Although the R_F values of the two monomethyl ethers were very similar the former was distinguished by its very slow and faint reaction with spray " a," whereas the latter behaved as a normal aldose towards this reagent. This phenomenon is characteristic of 2-ethers of aldoses (Kenner and Richards, personal communication) and will be discussed in detail later.

The neutralised solution was next de-ionised by stirring it with mixed Amberlite resins IR-120(H) and IR-4B(OH), and after filtration stirred for 1 hr. with Amberlite resin IRA-400 (borate form) (100 g.). The resulting solution was again de-ionised and evaporated to a colourless syrup (4·31 g.) which was shown by paper chromatography as above to be mainly 2-*O*-methyl-D-glucose, contaminated with a small amount of 2 : 3-di-*O*-methyl-D-glucose. A solution of the syrup in the minimum of boiling ethanol, when diluted with butan-1-ol (25 ml.) and kept at 0°, slowly deposited the crystalline 2-methyl ether (0·92 g.), showing m. p. 157—159° alone or in admixture with a sample prepared by the method of Weygand and Trauth (*loc. cit.*), $[\alpha]_D^{20}$ +65° (equil.; *c*, 1 in H_2O). A further quantity (0·88 g., total 12·5%) was obtained by evaporation of the liquors from the above crystallisation, followed by chromatography on powdered cellulose, with butan-1-ol followed by ethanol as eluant.

The p-*nitrophenylhydrazone*, prepared in the usual manner, showed m. p. 196—197° (Found : N, 12·9; OMe, 9·6. $C_{13}H_{19}O_7N_3$ requires N, 12·8; OMe, 9·4%).

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