1021. *The Separation of Isomeric Glycosides on Basic Ion-exchange Resins.*

By P. W. Austin, F. E. Hardy, J. G. Buchanan, and J. Baddiley.

A rapid, efficient means of resolving mixtures of isomeric glycosides is described. The method involves chromatography on a strongly basic anion-exchange resin in the hydroxide form. It has been employed for the separation of furanosides from pyranosides, α–β-mixtures of anomers, and a mixture of the methyl α-glycosides of two different hexoses.

Several methods have been developed in which ion-exchange resins are used for the separation of mixtures of neutral carbohydrates. In 1952 Roseman *et al.*[1] reported that when a mixture of reducing and non-reducing sugars was applied to a column of the strongly basic Amberlite IRA-400 resin in the hydroxide form and the column was washed rapidly with water, only the reducing sugars were retained; retention was much less marked when the resin was in the chloride, acetate, or carbonate form. Hough *et al.*[2] separated carbohydrates on columns of the strongly basic De-Acidite FF resin in the carbonate, hydrogen carbonate, or chloride form; by using large amounts of resin and eluting slowly with water a mixture of raffinose, sucrose, and glucose could be resolved. Mixtures of carbohydrates have also been resolved by chromatography on Dowex 1 resin in the borate form, the eluant being an aqueous solution of potassium tetraborate.[3,4] More recently Samuelson and Swenson [5] have used the sulphate form of this resin; with aqueous ethanol as eluant the separation of xylose, glucose, and maltose was achieved. Sulphonic acid resins [6,7] have also been used; thus, mixtures of oligosaccharides or methylated monosaccharides have been separated on columns of the lithium form of Dowex 50 resin, and mixtures of monosaccharides have been separated with the barium form of this resin.

In earlier work in this laboratory the hydroxide form of the strongly basic Dowex 1 resin was used for removing free sugars from mixtures with glycosides. A Koenigs–Knorr reaction between 3,4,6-tri-*O*-acetyl-*N*-4-methoxybenzylidene-α-D-glucosaminyl bromide and 1-*O*-benzoyl-5-*O*-benzyl-2,3-*O*-isopropylidene-D-ribitol gave, after removal of protecting groups, an α–β-mixture of the anomers of 4-*O*-D-glucosaminyl-D-ribitol together with ribitol and glucosamine; when an aqueous solution of the mixture was shaken for some time with this resin, and then filtered, and the resin was washed with a large volume of water, glucosamine was completely adsorbed.[8] Similarly, in order to remove glucose

[1] Roseman, Abeles, and Dorfman, *Arch. Biochem. Biophys.*, 1952, **36**, 232.
[2] Hough, Priddle, and Theobald, *Chem. and Ind.*, 1960, 900.
[3] Khym and Zill, *J. Amer. Chem. Soc.*, 1952, **74**, 2090.
[4] Zill, Khym, and Cheniae, *J. Amer. Chem. Soc.*, 1953, **75**, 1339.
[5] Samuelson and Swenson, *Acta Chem. Scand.*, 1962, **16**, 2056.
[6] Jones, Wall, and Pittet, *Canad. J. Chem.*, 1960, **38**, 2285.
[7] Jones and Wall, *Canad. J. Chem.*, 1960, **38**, 2290.
[8] Hardy, Buchanan, and Baddiley, *J.*, 1963, 3360.

from a mixture of glucose, glycerol, and the α- and β-anomers of 2-*O*-D-glucopyranosylglycerol, produced by a variation of the Koenigs–Knorr reaction (unpublished work), an aqueous solution of the mixture was applied to a column of Dowex 1 resin. Elution with water not only gave the non-reducing carbohydrates free from glucose but also separated them from each other; glycerol was eluted first, followed by 2-*O*-α-D-glucosylglycerol and then the corresponding β-anomer. Excellent separations of the α- and β-anomers of methyl D-glucopyranoside and the α- and β-anomers of methyl D-glucosaminide were also achieved by chromatography on this resin; in each case the α-anomer was eluted first. Moreover, the concentration of the glycosides in the eluate fractions was such that they could be detected by measurement of optical rotation and by paper chromatography without further concentration of the solutions.

A difficulty in the study of the Fischer glycoside synthesis [9] has been the resolution of the mixture of anomeric pyranosides and furanosides usually produced. Mowery and Ferrante,[10] in a study of the distribution of the isomers formed during the synthesis of methyl D-galactosides by the action of methanolic hydrogen chloride on D-galactose, separated β- from α-anomers but could not separate furanosides from pyranosides. These four isomers were separated by Augestad and Berner [11] by chromatography on a column of cellulose, but the method is rather tedious. This mixure can be resolved rapidly by chromatography on the hydroxide form of Dowex 1 resin. Water eluted the glycosides in the order: methyl α-D-galactopyranoside, methyl β-D-galactopyranoside, methyl α-D-galactofuranoside, methyl β-D-galactofuranoside. Each isomer was obtained in a high state of purity and any free galactose in the original mixture was retained by the resin; methyl β-D-galactofuranoside was not eluted from the resin until at least ten bed-volumes of water had been passed through the column. In a similar experiment a mixture of anomeric D-glucopyranosides and D-glucofuranosides containing a high proportion of the furanose forms, produced by means of the Fischer reaction, was chromatographed on the resin; the isomers were eluted in the same order as the isomeric D-galactosides, and pure samples of methyl α- and β-D-glucofuranoside were obtained.

The technique has also been used by others in these laboratories.[12] The separation of α- and β-anomers of methyl D-ribofuranoside and of α- and β-anomers of methyl D-ribothiapyranoside [13] has been achieved. In both separations the α- was eluted before the β-anomer, and the α-ribothiapyranoside crystallised for the first time. Moreover, the method is not restricted to the separation of α- and β-anomers of glycosides of a single sugar; a mixture of methyl α-D-galactopyranoside and methyl α-D-glucopyranoside was readily resolved.

Two other strongly basic anion-exchange resins have been examined for this type of separation. Permutit De-Acidite FF resin, with similar particle size and degree of cross-linkage to the Dowex 1 resin, behaved in exactly the same way and gave an excellent separation of methyl α- and β-D-glucopyranosides. However, the efficiency of a Dowex 2 resin of similar particle size but with a higher degree of cross-linkage was less marked; carbohydrates were less strongly adsorbed and only a partial separation of methyl α- and β-D-glucopyranosides was possible.

Ion-exchange chromatography on strongly basic resins is clearly a useful method for the separation of isomeric glycosides and is capable of wide application in the field of carbohydrate chemistry. Separations are rapid (5—15 hours), recoveries are high, and the only eluant required is water. In general, furanosides are adsorbed more strongly than pyranosides and in all the separations carried out so far, β-anomers are adsorbed more strongly than the corresponding α-anomers; free sugars are not eluted from the resin.

[9] Fischer, *Ber.*, 1893, **26**, 2400.
[10] Mowery and Ferrante, *J. Amer. Chem. Soc.*, 1954, **76**, 4103.
[11] Augestad and Berner, *Acta Chem. Scand.*, 1954, **8**, 252.
[12] Clayton and Hughes, personal communication.
[13] Clayton and Hughes, *Chem. and Ind.*, 1962, 1795.

Experimental

Paper Chromatography.—Whatman No. 1 or No. 4 paper was used with the descending solvent system butan-1-ol–ethanol–water–ammonia (*d* 0·88) (40 : 10 : 49 : 1).[14] Carbohydrates were detected by the periodate–Schiff reagents.[15]

Preparation of Resin Columns.—The ion-exchange resins were Dowex 1 (2% cross-linkages, 200—400 mesh, Cl^- form), Permutit De-Acidite FF (SRA 64) (2—3% cross-linkages, 200—400 mesh, Cl^- form), and Dowex 2 (8% cross-linkages, 200—400 mesh, Cl^- form). Resin was converted into the hydroxide form before use; Dowex 1 and 2 resins were washed with 2N-sodium hydroxide (5 bed-volumes) and then with water free from carbon dioxide until washings were neutral; the De-Acidite FF was washed with N-sodium hydroxide and then water. The resins were resuspended and allowed to settle slowly, mixtures were applied to the columns in small volumes of water, and elution was carried out with water free from carbon dioxide at a rate of 16—20 ml./hr. The resin could be used again if it was washed with N-hydrochloric acid (10 bed-volumes) and then water until washings were neutral, the chloride form then being treated as described above.

Separation of 2-O-α *and* -β-D-*Glucosylglycerols.*—A mixture of the two glucosides together with some D-glucose and glycerol was obtained by a Koenigs–Knorr reaction. The products ($R_{Glucose}$ 1·2) could not be separated by paper chromatography. The mixture (0·9 g.) in water (5 ml.) was applied to a column (18 × 1·7 cm.) of Dowex 1 (OH^- form) resin; elution with water and evaporation of fractions (15 ml.) gave glycerol (fraction 2), 2-*O*-α-D-glucosylglycerol (fractions 4—5), and 2-*O*-β-D-glucosylglycerol (fractions 6—8). The α-anomer (0·17 g.) was characterised as its hexabenzoate, m. p. 134°, $[\alpha]_D$ +96° (*c* 4·0 in $CHCl_3$); Charlson *et al.*[16] give m. p. 137—138°, $[\alpha]_D$ +96°. The β-anomer (0·07 g.) was characterised as its hexa-acetate, m. p. 127—129°, $[\alpha]_D$ −15° (*c* 7·2 in $CHCl_3$); Carter [17] gives m. p. 128°, $[\alpha]_D$ −15°.

Synthesis of Methyl α- *and* β-D-*Galactofuranosides by the Fischer Method.*—D-Galactose was treated with methanol containing a little sulphuric acid as described by Haworth *et al.*,[18] to give a mixture of methyl D-galactosides containing a high proportion of the furanose forms. This proportion was increased by extraction of the products with ethyl acetate; [18] the syrup (1·9 g.) obtained by evaporation of the ethyl acetate was dissolved in water (3 ml.) and chromatographed on a column (16 × 1·7 cm.) of Dowex 1 (OH^- form) resin. The eluate was collected in fractions (5 ml.), and the galactosides were eluted as shown in Table 1.

Table 1.

Elution of methyl D-galactosides from Dowex 1 (OH^- form) resin.

Fraction No.	Me galactoside	$R_{Glucose}$
8—9	α-D-Galactopyranoside (0·25 g.), m. p. 110° (monohydrate)	2·12
10	β-D-Galactopyranoside (0·1 g.), m. p. 177°	1·9
15—25	α-D-Galactofuranoside (0·35 g.), m. p. 91°, $[\alpha]_D$ +105° (*c* 3·0 in H_2O)	2·54
48—80	β-D-Galactofuranoside (1·05 g.), m. p. 68°, $[\alpha]_D$ −110° (*c* 3·0 in H_2O)	3·35

The anomeric furanosides were both crystallised from ethyl acetate; Augestad and Berner [11] give m. p. 91—92°, $[\alpha]_D$ +104°, for the α-anomer and m. p. 69°, $[\alpha]_D$ −112°, for the β-anomer. In a similar experiment a mixture of galactosides containing a high proportion of the pyranose forms, prepared by prolonged treatment of D-galactose with methanolic sulphuric acid, was successfully resolved into its components.

Synthesis of Methyl α- *and* β-D-*Glucofuranosides by the Fischer Method.*—D-Glucose (8 g.) was stirred with methanol (100 ml.) containing sulphuric acid (0·5 ml.) for 14 hr. at room temperature. Sulphuric acid (0·5 ml.) was again added and stirring was continued for a further 10 hr. After neutralisation with barium carbonate and filtration, the solution was evaporated to dryness. The residue was extracted with hot ethyl acetate (5 × 60 ml.), the extract cooled, clarified (K_2CO_3), filtered, and evaporated to a syrup (1·5 g.). Paper chromatography showed

[14] Hirst, Hough, and Jones, *J.*, 1949, 928.
[15] Baddiley, Buchanan, Handschumacher, and Prescott, *J.*, 1956, 2818.
[16] Charlson, Gorin, and Perlin, *Canad. J. Chem.*, 1956, **34**, 1811.
[17] Carter, *Ber.*, 1930, **63**, 1684.
[18] Haworth, Hirst, Jones, and Woodward, *J.*, 1938, 1575.

the presence of the anomeric glucofuranosides, together with small amounts of the anomeric pyranosides, and glucose. A solution of the syrup in water (4 ml.) was chromatographed on a column (25 × 1·7 cm.) of Dowex 1 (OH^- form) resin. The eluate was collected in fractions (5 ml.), and the glucosides were eluted as shown in Table 2.

TABLE 2.

Elution of methyl D-glucosides from Dowex 1 (OH^- form) resin.

Fraction no.	Me glucoside	$R_{Glucose}$
10—11	α-D-Glucopyranoside (0·07 g.), m. p. 167°	2·3
12—14	β-D-Glucopyranoside (0·05 g.), m. p. 108°	2·1
21—39	α-D-Glucofuranoside (0·45 g.), m. p. 63°, $[\alpha]_D$ +115° (*c* 2·0 in H_2O)	3·05
39—60	β-D-Glucofuranoside (0·7 g.), $[\alpha]_D$ −78° (*c* 3·0 in H_2O)	3·25

Methyl β-D-glucofuranoside was characterised as its tetra(phenylcarbamoate), m. p. 217°. Phillips [19] gives m. p. 218—219° for this derivative, $[\alpha]_D$ −77° for methyl-β-D-glucofuranoside, and m. p. 60—62°, $[\alpha]_D$ +110°, for methyl α-D-glucofuranoside.

Separation of Methyl α- and β-D-Glucosaminides.—Equal amounts (0·1 g.) of the hydrochlorides of the two anomers were mixed. On paper chromatography the mixture ran as one spot ($R_{Glucosamine}$ 1·65). This material in water (2 ml.) was applied to a column (25 × 1·7 cm.) of Dowex 1 (OH^- form) resin and elution was carried out with water. The first 50 ml. of eluate were discarded and then fractions (2·5 ml.) were collected. Fractions 4—6 contained methyl α-D-glucosaminide characterised as its hydrochloride (0·085 g.), m. p. 190—192°, $[\alpha]_D$ +145° (*c* 5·0 in H_2O), while fractions 8—10 contained methyl β-D-glucosaminide, also characterised as its hydrochloride (0·085 g.), m. p. 191—192°, $[\alpha]_D$ −26° (*c* 5·0 in H_2O).

Separation of Methyl α-D-Glucopyranoside and α-D-Galactopyranoside.—Equal amounts (0·15 g.) of these glycosides were chromatographed on a column (25 × 1·7 cm.) of Dowex 1 (OH^- form) resin. When the column had been washed with water (50 ml.) the eluate was collected in fractions (5 ml.). Fractions 3—5 contained pure methyl α-D-galactoside (0·13 g.), m. p. 110°; fraction 6 gave material (0·01 g.) shown by paper chromatography to contain both glycosides; fractions 7—9 contained pure methyl α-D-glucoside (0·13 g.), m. p. 167°.

Comparison of Chromatographic Properties of the Hydroxide Forms of Dowex 1 and 2 and Permutit De-Acidite FF.—Columns (25 × 1·7 cm.) of each resin were prepared and mixtures (0·5 g.) containing equal amounts of methyl α- and β-D-glucopyranoside were applied in aqueous solution. After each column had been washed with water (30 ml.) the eluates were collected in fractions (5 ml.). With Dowex 1, fractions 7—10 contained methyl α-D-glucoside, and fractions 11—16 contained methyl β-D-glucoside; De-Acidite FF behaved in the same way. With Dowex 2, however, the glycosides were in fractions 1—7 and only a partial separation was achieved.

This work was carried out during the tenure by one of us (F. E. H.) of an I.C.I. Fellowship and by another (P. W. A.) of a D.S.I.R. Studentship. We thank the Department of Scientific and Industrial Research and the Nuffield Foundation for financial support.

DEPARTMENT OF ORGANIC CHEMISTRY,
THE UNIVERSITY, NEWCASTLE UPON TYNE, 1.

[*Received, May 21st,* 1963.]

[19] Phillips, *J. Amer. Chem. Soc.*, 1954, **76**, 3598.