Separation of glycopyranosides on ion-exchange resins

Reaction of methyl glycopyranosides with methanesulfonyl chloride in N,N-dimethylformamide (DMF) causes selective replacement of the primary hydroxyl group by chlorine in high yield[1]. The resulting methyl 6-chloro-6-deoxy-glycopyranosides are reduced quantitatively by lithium aluminum hydride in boiling tetrahydrofuran to methyl 6-deoxy-glycopyranosides[1]. These reactions provide an improvement over current syntheses[2–4] of methyl 6-chloro-6-deoxyglycopyranosides and methyl 6-deoxy-glycopyranosides in both yield and number of steps involved.

While examing a possible mechanism[5] for the introduction of chlorine with methanesulfonyl chloride in DMF, the reaction was performed in the presence of lithium chloride. On removing the latter compound, in aqueous solution, on a column of Rexyn 300 mixed bed ion-exchange resin, it was observed that partial separation of methyl 6-chloro-6-deoxy-glucopyranoside and unchanged methyl glucopyranoside occurred. This paper describes some separations of glucopyranosides on columns of anion-exchange resin in the hydroxide or chloride form with water or 75 % (v/v) aqueous 1-propanol as eluant.

Experimental

The α- and β-anomers of methyl 6-deoxy-D-glucopyranoside (I) and of methyl 6-chloro-6-deoxy-D-glucopyranoside (II) were prepared by the methods indicated above from the corresponding anomeric methyl glucopyranoside (III) obtained from Pfanstiehl Laboratories, Waukegan, Ill. Phenyl α- and β-D-glucopyranosides were obtained from Sigma Chemical Company, St. Louis, Mo.

Rexyn 300 and Dowex 1 X2 (200–400 mesh) were obtained from Fisher Scientific Company.

Mixtures of glycosides (100 μ moles of each component) were applied to columns (28 × 1 cm) of Dowex 1 X2 resin (Cl^- or OH^-) which had been packed in water or 75 % (v/v) aqueous 1-propanol. Elution was effected with the solvent used to pack the column and the eluate was monitored continuously with an ETL-NPL automatic polarimeter (The Bendix Corporation, Cincinnati, Ohio) connected to a Leeds-Northrup Speedomax "H" AZAR recorder. Fractions (1 ml) were collected, using a flow rate of 0.1 ml/min.

Ascending thin layer chromatography (TLC) of the fractions was performed on layers of "Silica Gel G according to STAHL" (distributed by Brinkmann Instruments, Inc., Great Neck, Long Island, N.Y.) with ethyl acetate–ethanol–water (15:2:1, by vol.) as the developing solvent. Glycosides were located by spraying with a 1 % solution of α-naphthol in ethanol followed by 10 % sulfuric acid then heating at 120°.

Gas–liquid chromatographic (GLC) examination of the glycoside fractions as their trimethylsilyl ethers, prepared as described by SWEELEY and co-workers[6], was performed with a Wilkens Aerograph Model A-700 ("Autoprep"), equipped with a column (6 ft. × 1/4 in.) of 10 % Carbowax 6000 on 80–100 mesh Chromosorb W at 145°, and a flame ionization detector. Nitrogen was used as the carrier gas at a flow rate of 120 ml/min.

Results and discussion

The automatic polarimeter provides a convenient and sensitive method of

following the elution of glycosides from resin columns, and the anomeric nature of an eluted compound is indicated by the sign of rotation. The components of the individual fractions are rapidly identified by TLC, the R_F values for I–III being 0.48, 0.37, and 0.16, respectively. Except with phenyl glucopyranosides, our mixtures containing α- and β-anomers are not resolved by TLC; the α-anomer of phenyl glucopyranoside has R_F 0.37, and the β-anomer R_F 0.43.

Identification of eluted components was also performed by GLC. This method is particularly useful for the separation of anomers. Of a variety of column packings tested, 10 % Carbowax 6000 on 80–100 mesh Chromosorb W gave the best separations. The results are shown in Table I.

TABLE I

GAS CHROMATOGRAPHY OF TRIMETHYLSILYL ETHERS OF GLYCOSIDES

Column: 10 % Carbowax 6000 on 80–100 mesh Chromosorb W, 6 ft. × 1/4 in.
Conditions: Column 145°; nitrogen carrier 120 ml/min; flame ionization detector.

Silylated glycoside	*Elution time (min)*
Methyl 6-deoxy-α-D-glucopyranoside	3.4
Methyl 6-deoxy-β-D-glucopyranoside	3.6
Methyl α-D-glucopyranoside	7.0
Methyl β-D-glucopyranoside	7.5
Methyl 6-chloro-6-deoxy-α-D-glucopyranoside	11.9
Methyl 6-chloro-6-deoxy-β-D-glucopyranoside	10.2

The initial observation that III emerged earlier than II from a column of the mixed bed resin Rexyn 300 using water as eluant led to a study of separations on the individual components of the Rexyn 300. It was found that separation of the glycoside mixture was effected by the strong base resin component. AUSTIN and co-workers[7] have used Dowex 1 X2 (OH^-, 200–400 mesh) to separate the four isomeric methyl glycosides derived from D-glucose or D-galactose with water as the eluant. Following this procedure[7], separations were attempted of mixtures of α- and/or β-anomers of I, II and III (see Table II). With water as eluant, I and III are incompletely separated but are readily separated from II. However, when 75 % (v/v) aqueous 1-propanol is used as eluant, mixtures of I, II and III are readily resolved except that we were unable to separate the anomers of II. In both solvents I is eluted first but the order of elution of II and III depends on the solvent used. DEKKER[8] has shown that separations of nucleosides on strong base resins depend on the acidity of sugar hydroxyl groups, and differences in acidity of the various hydroxyl groups of carbohydrates have been indicated by polarographic[9], conductometric[9], and titrimetric methods[10]. It is apparent from the different elution order of II and III with different solvents that factors other than sugar hydroxyl acidity can be important in separations on a resin in the hydroxide form. SAMUELSON and co-workers[11,12] have shown that the sorption of polar nonelectrolytes on resins from aqueous ethanol solution is due mainly to their higher solubility in the water-rich resin phase than in the less polar aqueous ethanol phase. This partition method has been applied[13] to the separation of reducing sugars on Dowex 1,

TABLE II

CHROMATOGRAPHY OF GLYCOSIDES ON DOWEX 1 (200–400 MESH) RESIN

Glycoside	*Peak elution volume (ml)*[a]			
	75% (v/v) aqueous 1-propanol eluant		*Water eluant*	
	Resin OH^- form	*Resin Cl^- form*	*Resin OH^- form*	*Resin Cl^- form*
Methyl 6-deoxy-α-D-glucopyranoside	28	26	60[b]	22
Methyl 6-deoxy-β-D-glucopyranoside	34	—	66[b]	—
Methyl 6-chloro-6-deoxy-α-D-glucopyranoside	39	38	98	30
Methyl 6-chloro-6-deoxy-β-D-glucopyranoside	39	—	98	—
Methyl α-D-glucopyranoside	60	64	74[b]	18
Methyl β-D-glucopyranoside	82	—	82	—
Phenyl α-D-glucopyranoside	32	38[b]	—	—
Phenyl β-D-glucopyranoside	53	45	—	—

[a] Total volume of eluant (ml) at maximum concentration of eluted component.
[b] Overlapping with compound eluted next.

the sulfate form being used since reducing sugars are retained by the hydroxide form[14]. Separations of the α- or β-anomeric mixtures of I, II and III on the chloride form of Dowex 1 X2 (200–400 mesh) are shown in Table II. The elution order of I–III with 75% aqueous 1-propanol as eluant is the same with the chloride or hydroxide form of the resin, indicating that separation is due primarily to a partition process[11]. The distribution coefficient (concentration in resin/concentration in external solution) should decrease as the substituent at C-5 of the pyranose ring changes in the order $-CH_2OH > -CH_2Cl > -CH_3$, and this is in accord with the observed elution order.

The order of elution with water as eluant depends on the nature of the resin counter-ion and is different from the order expected on the basis of partition coefficients. A possible explanation of the elution order is that sorption is mainly by ion-exchange with the resin in the hydroxide form[8]. However, the sorption with the chloride form probably involves van der Waals and polar interactions[15]. Of compounds I–III, van der Waals attraction to the hydrocarbon matrix of the resin would be expected to be greatest with I through its methyl substituent at C-5. The polar interactions of compounds I–III with the quaternary ammonium groups of the resin will be influenced by the nature of the substituent at C-5. Further, the substituent at C-5, by its inductive effect, will influence the electron density at the pyranose ring oxygen and will modify the polar interactions of this atom.

The phenyl D-glucopyranosides are not sufficiently soluble in water to use water as eluant. With 75% (v/v) aqueous 1-propanol as eluant, complete separation of the anomers is obtained with Dowex 1 X2 (200–400 mesh) in the hydroxide form but only partial separation occurs with the chloride form (Table II).

Separations scaled up to 1 g of glycoside mixture have been performed on columns (75 × 3 cm) of Dowex 1 X2 (OH^-, 200–400 mesh) with 75% (v/v) aqueous 1-propanol. The resin column may be used repeatedly and recoveries are almost quantitative.

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