Carbohydrate Research, 51 (1976) 149–155

THE SEPARATION OF METHYL ETHERS OF METHYL 2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSIDES ON A STRONGLY BASIC ION-EXCHANGE RESIN

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(Received January 13th, 1976; accepted for publication, April 14th, 1976)

ABSTRACT

The methyl ethers of methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside can be isolated on a preparative scale by chromatography on Dowex-1(HO^-) resin. This procedure greatly simplifies the purification of methyl ethers, and has been used to isolate the methyl ethers produced by partial methylation of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside. The separations are thought to depend on an ion-exchange process in which all the free hydroxyl groups are involved. It is concluded that the following acidity sequence holds: HO-4 > HO-3 > HO-6.

INTRODUCTION

The chromatography of glycosides on strongly basic ion-exchange resins in the hydroxide form is well-established[1–3] and is applicable to the preparation of anomers on a gram scale[3]. The fractionation of various glycosides was assumed to be due to an ion-exchange process involving ionisation of one or more of the hydroxyl groups and the separation of anomers due to electrostatic interactions.

We now report the preparation and isolation of various methyl ethers of methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside, and offer an explanation of their behaviour in terms of the ionisation of hydroxyl groups which involves some modification of the earlier proposals[3]. A brief account of some of the results has already been given[4].

EXPERIMENTAL

Analytical methods. — Optical rotations were determined on a Perkin–Elmer 141 polarimeter at 25° using a 1-dm cell. Column eluates were monitored for optical rotation at 365 nm using a 1-cm pathlength, or, for u.v. absorption at 227 nm. The retention times of the hexosamines were determined on the 28-cm column of a

Locarte Mini Amino Acid Analyser eluted with the pH 5.28 (0.35M Na^+) buffer described by Moore and Stein[5]. The elution times of the hexosamines were compared with those of authentic samples supplied by Dr. A. S. R. Donald of the Lister Institute of Preventive Medicine, London, S.W.1.

The degree of methylation of derivatives of 2-amino-2-deoxyglucose was determined by chromatography on Whatman 3MM paper with 1-butanol–acetic acid–water (4:1:1). The monomethyl ethers had a mobility relative to 2-amino-2-deoxyglucose (R_{GlcNH_2}) of 1.45, and dimethyl ethers had R_{GlcNH_2} 2.10.

Chromatography of glycosides. — A column of Dowex-1(HO^-) resin was prepared and eluted as described by Neuberger and Wilson[3]. Traces of acids or reducing sugars were first removed by using a column (20 × 5 cm) of Deacidite FF (HO^-) resin (Permutit Ltd., London) and elution with water; all fractions that showed optical activity were combined and concentrated. The optically active products were then applied to a column (60 × 3 cm) of Dowex-1(HO^-) resin (2% cross-linked, 200–400 mesh) and eluted with deionised, distilled, carbon dioxide-free water at 30 ml/h. Elution volumes are given in Table I.

TABLE I

ELUTION VOLUMES OF DERIVATIVES OF METHYL GLUCOPYRANOSIDES ON A COLUMN OF DOWEX-1(HO^-) RESIN

Location of methyl group		*Elution volume (ml)*		$V/V_P - I$[c]	
	Group at C-2	*α anomer*[a]	*β-anomer*	*α anomer*	*β anomer*
	NHAc	700	820[a]	1.22	1.60
3	NHAc	510	610[a]	0.62	0.94
4	NHAc	430	460	0.37	0.46
6	NHAc	615	700	0.95	1.22
3,4	NHAc	345	365	0.10	0.16
3,6	NHAc	n.d.[b]	540	n.d.	0.71
4,6	NHAc	n.d.	415	n.d.	0.32
3,4,6	NHAc	315	325[a]	0	0.03
2,3,4,6	OMe	315	n.d.	0	n.d.

[a]Compounds prepared by standard methods. The other compounds were prepared by a partial methylation of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (see Experimental). [b]Not determined. [c]Increase in elution volume (V) relative to that (V_P) of permethylated D-glucose.

Preparation of glycosides. — (*a*) *Methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides.* The glycosides were prepared by heating 2-acetamido-2-deoxy-D-glucose with methanol and Dowex-50(H^+) resin under reflux conditions. The anomers were isolated by chromatography on Dowex-1(HO^-) resin[3].

(*b*) *Methyl 2-acetamido-2-deoxy-3-O-methyl-α-D-glucopyranoside.* This compound was prepared by the method of Neuberger[7] but, instead of purifying the product by repeated crystallization, the contaminant (original glycoside, 5%) was removed by chromatography on Dowex-1(HO^-) resin as described above.

(c) *Methyl 2-acetamido-2-deoxy-4-O-methyl-α-D-glucopyranoside and methyl 2-acetamido-2-deoxy-3,4-di-O-methyl-α-D-glucopyranoside.* These compounds were prepared by the literature procedure[8,9], except that the trityl group was removed immediately after the methylation step and the methyl ethers were isolated by chromatography on Dowex-1(HO^-) resin (see above). Three products were obtained (Fig. 1), as expected, together with a trace of unreacted glycoside. The ethers were identified as the free amino sugars by p.c. and elution time on the amino acid analyser after hydrolysis (4M HCl, 4 h, 100°) (Table II).

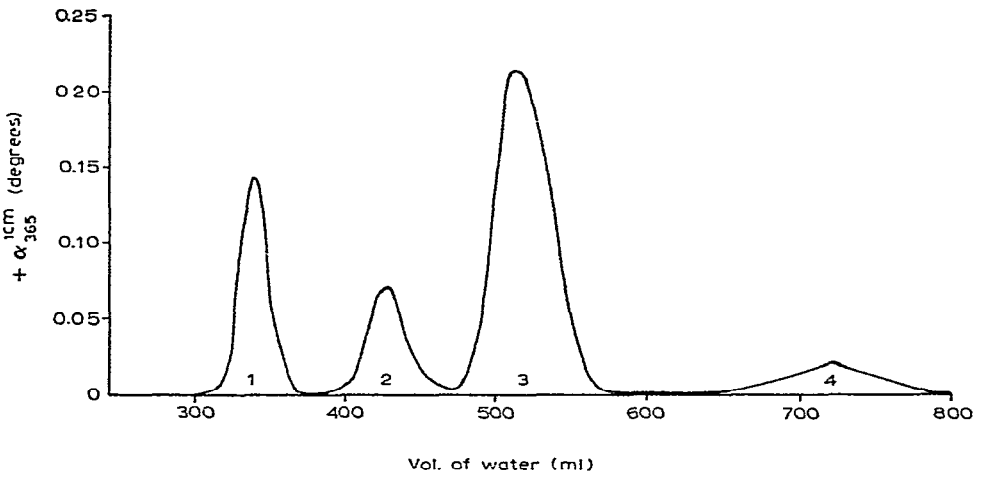

Fig. 1. Elution pattern on Dowex-1 (HO^-) resin of methyl ethers of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside. Peak 1, 3,4-di-*O*-methyl; peak 2, 4-*O*-methyl; peak 3, 3-*O*-methyl; peak 4, parent glycoside.

TABLE II

THE ELUTION TIMES (R_{GlcNH_2}) RELATIVE TO THAT OF 2-AMINO-2-DEOXYGLUCOSE ON THE AMINO ACID ANALYSER OF AUTHENTIC SAMPLES OF METHYL ETHERS OF 2-AMINO-2-DEOXYGLUCOSE AND OF THE PRODUCTS OF ACID HYDROLYSIS OF THE METHYL ETHERS OF 2-ACETAMIDO-2-DEOXY-αβ-D-GLUCOSIDE.

Location of methyl groups	R_{GlcNH_2}		
	Authentic sample	*Products of hydrolysis of*	
		Methyl α-glycoside	*Methyl β-glycoside*
3	0.94	0.93	0.97
4	1.05	1.02	1.05
6	1.10	1.10	1.09
3,4	1.50	1.46	1.52
3,6	1.01	n.d.	1.04
4,6	1.29	n.d.	1.29
3,4,6	1.73	n.d.	1.80

(*d*) *Methyl 2-acetamido-2-deoxy-6-*O*-methyl-α-*D*-glucopyranoside.* This compound, prepared by the method of Jeanloz[10], contained some methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, which could be removed by chromatography on Dowex-1(HO^-) resin. After acid hydrolysis, the product behaved as a monomethyl ether on p.c. and had the expected elution time on the amino acid analyser (Table II).

(*e*) *Methyl 2-acetamido-2-deoxy-3-*O*-methyl-β-*D*-glucopyranoside.* This compound was prepared from methyl 2-acetamido-2-deoxy-β-D-glucopyranoside by the method described above for the α anomer, and purified by chromatography on Dowex-1(HO^-) resin (Table I).

(*f*) *Methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-α- and β-*D*-glucopyranosides.* These compounds were prepared by a modification of the method of Neuberger[11]. 2-Acetamido-2-deoxy-αβ-D-glucopyranoside was methylated with methyl sulphate in alkali. The reaction mixture was extracted with chloroform, and the extract was washed with water and concentrated. The residue was crystallised from chloroform–light petroleum (b.p. 60–80°) to give first the β anomer, followed by an αβ-mixture. Concentration of the mother liquor gave the α anomer, which was recrystallized from ethyl acetate.

When the fractions were analysed separately on Dowex-1(HO^-) resin, only one peak of material absorbing at 227 nm was found, but the optical rotations showed that the first fraction was mainly the β anomer and the last fraction mainly the α anomer. Fractions that showed the highest rotations were combined and concentrated to give the α anomer, m.p. 151°, $[\alpha]_D$ +104° (*c* 1, water), +109° (*c* 1, chloroform); lit.[11]: m.p. 151°, $[\alpha]_D$ +102° (water); β anomer, m.p. 193°, $[\alpha]_D$ −14° (*c* 2, water), +25° (*c* 2, chloroform); lit.[11,12]: m.p. 192°, $[\alpha]_D$ +19.5° (chloroform), +20.3° (chloroform).

When a 1:2 αβ-mixture was analysed on two large columns of Dowex-1(HO^-) resin (bed volumes, 480 and 920 ml), the anomers were only just resolved by measuring optical rotation, but were not resolved by measuring u.v. absorption.

*Partial methylation of methyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside and fractionation of the products.* — To a solution of the title glycoside (1.2 g, 5 mmol) in water (10 ml) at 65°, 30% aqueous (w/v) sodium hydroxide (2 ml) and methyl sulphate (1 ml, 10 mmol) were added. The mixture was stirred at 65° for 2 h, then cooled, neutralised (1.5M H_2SO_4), and concentrated to dryness, and the residue was dried over P_2O_5 *in vacuo*. The powder was suspended in ethanol, Na_2SO_4 was removed by centrifugation and washed twice with ethanol, and the combined supernatants were concentrated to dryness at 40°. A solution of the product in water (10 ml) was applied to a column (40 × 3 cm) of Dowex-50(H^+) resin and eluted with water. Sulphuric acid was eluted from 90–150 ml and the glycosides from 200–300 ml. The glycoside fraction was concentrated and the glycosides were fractionated on analytical columns (see Fig. 2). This methylation procedure would be expected to produce a mixture of seven methyl ethers; peak 5 was in the position of authentic methyl 2-acetamido-2-deoxy-3-*O*-methyl-β-D-glucopyranoside, and peak 7 was the starting glycoside. Since authentic samples of the other methyl ethers were not available for comparison,

material from each of the peaks was hydrolysed (4M HCl, 100°, 3 h) and the free amino derivatives were identified by p.c. and by their elution times on the amino acid analyser (see Table II).

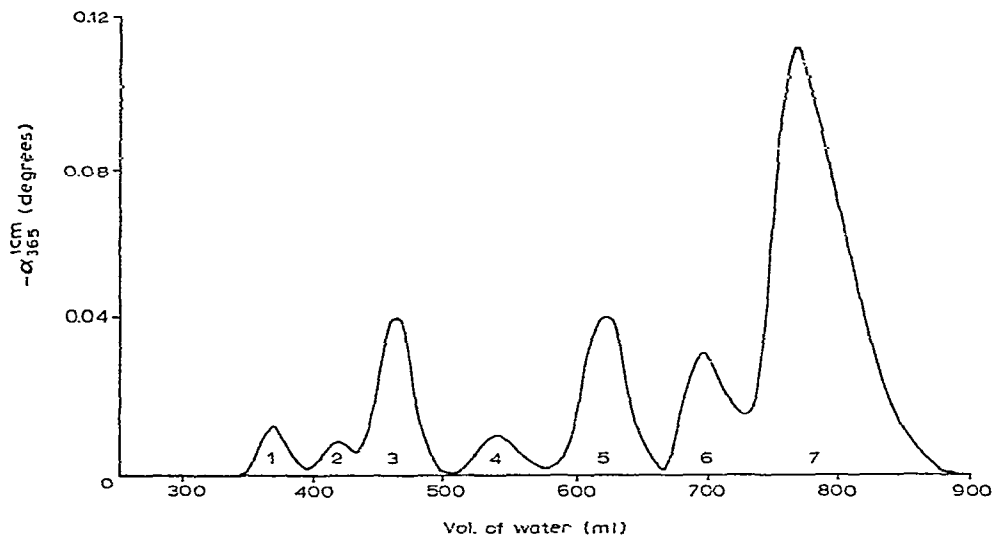

Fig. 2. Elution pattern on Dowex-1 (HO^-) resin of methyl ethers of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside. Peak 1, 3,4-di-*O*-methyl; peak 2, 4,6-di-*O*-methyl; peak 3, 4-*O*-methyl; peak 4, 3,6-di-*O*-methyl; peak 5, 3-*O*-methyl; peak 6, 6-*O*-methyl; peak 7, parent glycoside.

As expected, the amounts of the monomethyl ethers (peaks 3, 5, and 6) were much greater than the amounts of the dimethyl ethers (peaks 1, 2, and 4). Traces of the trimethyl ether were eluted with the 3,4-di-*O*-methylglycoside in peak 1. The elution volumes for these methyl β-pyranosides are given in Table I, together with the elution volumes of the α anomers. The order of elution of a series of methyl β-glycosides should be the same for the corresponding series of α anomers, and this behaviour was found (Table I). An attempt to separate the products of a similar partial methylation of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside was not completely successful with the 60-cm column, but a better separation should be possible on a longer column of Dowex-1(HO^-) resin.

DISCUSSION

The separation of a variety of methyl ethers of 2-acetamido-2-deoxy-D-glucosides described herein is another example of the high resolving power of Dowex-1(HO^-) resin which has been demonstrated for a variety of α and β glycosides[1,2,3,14] and also for the methyl ethers of adenosine[15]. Published procedures for the preparation of methyl ethers of 2-acetamido-2-deoxyglucosides can be considerably simplified by the use of this resin, since the purification of the reaction products by chromato-

graphy eliminates the necessity of repeated recrystallisation. Furthermore, the components of a mixture of mono- and di-methyl ethers produced by partial methylation of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside could also be separated on this resin.

The separation of glycosides on Dowex-1(HO^-) resin is likely to be an ion-exchange process dependent on the ionisation of hydroxyl groups, since glycosides are known to behave as weak polybasic acids in aqueous solution. We therefore assume that the order of elution of glycosides indicates their relative acidities. This is emphasised by the observations[16] that methyl 2-amino-2-deoxyglucofuranosides, which are much more acidic than the corresponding pyranosides when measured by pH titration, are retarded on Dowex-1(HO^-) resin to a greater extent than are the pyranosides. Methyl 2-acetamido-2-deoxyglucofuranosides were retarded more than the corresponding pyranosides.

Consideration of the acidity of unsubstituted methyl glycosides led Neuberger and Wilson[3] to explain the separation of anomeric pairs in terms of a predominant ionisation of HO-2. For the 2-acetamido-2-deoxypyranosides, it was suggested that HO-3 might most easily lose a proton, although the ionisation of the acetamido group could not be ruled out. From the results reported herein, it appears that the acetamido group does not ionise to any significant extent since the permethylated methyl α-D-glucoside, which lacks an acetamido group, and the permethylated methyl 2-acetamido-2-deoxy-α-D-glucoside were eluted from the column in the same volume and were not retarded significantly.

From the behaviour of the monomethylated derivatives, we suggest that all three hydroxyl groups of the 2-acetamido-2-deoxyglucosides can ionise to some extent when in contact with Dowex-1(HO^-) resin, because methylation at any position reduced the elution volume. Since methylation of HO-6 has the smallest effect on the elution volume, we conclude that the HO-6 is the least acidic. This is supported by the observation that the 3,4-di-*O*-methylglycosides in which only HO-6 is free are only slightly more retarded than the 3,4,6-tri-*O*-methylglycosides (Table I). Similarly, since the 3-*O*-methylglycosides are eluted later than the 6-*O*-methyl derivatives, we conclude that HO-3 is more strongly ionised than HO-6. Hence, HO-4 must be the most acidic hydroxyl in the unsubstituted glycoside, since its methylation has the greatest effect on elution position. This conclusion is supported by the behaviour of the di-*O*-methyl derivatives, since the 4,6-di-*O*-methyl derivative, with HO-3 free, is eluted before the 3,6-di-*O*-methyl derivative with HO-4 free. Similar reasoning was used by Gin and Dekker[15] to deduce the acidities of the various hydroxyl groups of adenosine.

The importance of HO-4 is shown by the observation[3] that pyranoid derivatives of galactose and glucose, which differ only in the orientation of HO-4, differ considerably in their behaviour on columns of Dowex-1(HO^-) resin; glucosides and 2-acetamido-2-deoxyglucosides (HO-4 equatorial) are retarded more than the galactosides and 2-acetamido-2-deoxygalactosides (HO-4 axial). These results thus indicate a greater acidity for the *eq*HO-4 than for *ax*HO-4 and *eq*HO-3. These data suggest

that *eq*HO-4 is more accessible for interaction either with solvent or with the quaternary ammonium groups of the Dowex-1 resin than *ax*HO-4 or *eq*HO-3.

For all the pairs of compounds listed in Table I, the methyl α-glycosides are eluted before the β anomers. The separation of the α and β anomers is largest for the unsubstituted glycosides and almost non-existent for the fully methylated glycosides. It is most noticeable that substitution of HO-4 markedly reduces the magnitude of this separation of the anomers.

ACKNOWLEDGMENTS

We thank the Medical Research Council and the Wellcome Trust for financial support. This work was begun when the authors were in the Department of Chemical Pathology, St. Mary's Hospital Medical School, London, W.2.

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