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Note

Resolution of anomeric 2-amino-2-deoxy-D-glycofuranosides and -glycopyranosides by cation-exchange chromatography[1]

SHIGEHIRO HIRANO AND MAKOTO ISHIGAMI
Department of Agricultural Biochemistry, Tottori University, Tottori 680 (Japan)
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The resolution of anomeric 2-acetamido-2-deoxy-D-glycofuranosides and glycopyranosides has been effected by differential solubility, by chromatography on cellulose, charcoal–Celite, starch, *etc*, or, more recently, by anion-exchange chromatography[2–7] We now report another convenient method, based on cation-exchange chromatography (see Experimental)

2-Amino-2-deoxy-D-glycosides were produced in 83–96% yield by *N*-deacetylation of 2-acetamido-2-deoxy-D-glycosides

Distinctly different retention times (R_{NH_3}) were observed (Table I) for the anomeric 2-amino-2-deoxy-D-glycofuranosides and -glycopyranosides having the

TABLE I

RETENTION TIMES (R_{NH_3}) OF THE ANOMERS OF 2-AMINO-2-DEOXY-D-GLYCOSIDES RELATIVE TO AMMONIA, AS DETERMINED BY AN AMINO ACID ANALYZER

2-Amino-2-deoxy-D-glycoside[a]	R_{NH_3}		$\Delta R^{d}_{NH_3}$
	β	α	
Methyl 2-amino-2 deoxy-D glucopyranoside[b]	0 43	0 51	0 08
Methyl 2-amino-2-deoxy-D galactopyranoside[b]	0 47	0 51	0 04
Methyl 2-amino-2-deoxy-D-mannopyranoside[b]	0 50	0 65	0 15
Ethyl 2-amino-2-deoxy-D-glucopyranoside[b]	0 47	0 56	0 09
Ethyl 2-amino-2-deoxy D-galactopyranoside	0 52	0 58	0 06
Ethyl 2-amino-2-deoxy-D-mannopyranoside	0 56	0 75	0 19
Phenyl 2-amino-2 deoxy-D-glucopyranoside	1 93	2 63	0 70
Phenyl 2-amino-2 deoxy-D-galactopyranoside	1 93	2 55	0 62
Phenyl 2 amino-2-deoxy-D-mannopyranoside	2 26	4 86	2 60
Methyl 2 amino-2-deoxy D glucofuranoside[c]	0 50	0 62	0 12
Methyl 2-amino-2-deoxy-D galactofuranoside[c]	0 55	0 51	−0 04
Methyl 2-amino-2-deoxy-D-mannofuranoside[c]	0 62	0 54	−0 08
Ethyl 2 amino-2-deoxy D-galactofuranoside	0 62	0 57	−0 05
Ethyl 2-amino 2-deoxy-D-mannofuranoside	0 70	0 61	−0 09

[a]*N*-Acetyl derivatives of these 2 amino-2-deoxy D glycosides were prepared by the corresponding conventional method [b]See Ref 16 [c]The *N* acetyl derivatives were kindly supplied by Dr H Kushida, Kyoto General Medico Chemical Laboratory Kyoto [d]R_{NH_3} of the α anomer − R_{NH_3} of the β anomer

galacto, *gluco*, and *manno* configurations For the D-galacto-, D-gluco-, and D-manno-pyranosides and D-glucofuranosides, the α anomer was eluted after the β anomer, but the reverse was found for the D-galacto- and D-manno-furanosides

The values of R_{NH_3} and ΔR_{NH_3} for pairs of α and β anomers increased in the order phenyl > ethyl > methyl, and were larger for D-mannopyranosides (axial amino group) than for the D-galactopyranosides and -glucopyranosides (equatorial amino group)

D-Galactopyranosides (axial HO-4) showed relatively small values of ΔR_{NH_3} in comparison with D-glucopyranosides and D-mannopyranosides

The foregoing elution patterns, which were determined on an amino acid analyzer, can be used for preparative separations (Table II)

TABLE II

2-AMINO-2 DEOXY D-GLYCOSIDES OBTAINED FROM THE *N*-ACETYL GLYCOSIDES PREPARED BY THE FISCHER AND HELFERICH METHODS

2-Amino 2-deoxy-D glycoside	*Preparation method*[b]	*Pyranoside (%)*[a]		*Furanoside (%)*[a]	
		α	β	α	β
Ethyl 2-amino-2-deoxy D glucoside[c]	F	53 (43)	47 (40)	—[d] (0)	—[d] (0)
Phenyl 2-amino-2-deoxy-D-glucoside	H	58	42	—[d]	—[d]
Ethyl 2-amino-2 deoxy D mannoside	F	78	12	6	4
Ethyl 2 amino 2-deoxy-D galactoside	F	78[e]	16		6
Methyl 2 amino 2 deoxy D galactoside[f]	F	75[e] (35)	16		9

[a]Ratios obtained by an amino acid analyzer, the numbers in the brackets are the yields of hydrochlorides isolated by the preparative method [b]F = the Fischer method, H = the Helferich method [c]α Pyranoside HCl, m p 199–203°, $[\alpha]_D^{23}$ +135° (*c* 0 48 water) β-pyranoside HCl, m p 227–228 5 , $[\alpha]_D^{24}$ −18 7 (*c* 0 48, water) [d]Not detected by an amino acid analyzer [e]Also includes the α-furanoside [f]The α- and β-pyranosides and the α furanoside were overlapped in preparative chromatography For resolution the 2 acetamido-2-deoxy D galactoside was separated into two fractions on a column (2 × 30 cm) of Dowex-1 X8 (OH^-) resin eluted with distilled water The first fraction was positive for the chromotropic acid reaction, and gave products having R_{NH_3} 0 57 (α furanoside) and 0 62 (β-furanoside) on *N* deacetylation The second fraction gave a negative chromotropic acid reaction, and gave products having R_{NH_3} 0 52 (β-pyranoside) and 0 58 (α pyranoside) on *N*-deacetylation Each of the anomers was isolated by cation-exchange chromatography (see Experimental), the α-pyranoside HCl had m p 216–219 5 , n m r data (D_2O) δ 4 95 (d, 1 H, $J_{1,2}$ 4 0 Hz, H-1), 1 10 (t, 3 H, CH_3)

A solution of each of the anomers of ethyl 2-acetamido-2-deoxy-D-glucopyranoside, prepared from the corresponding ethyl 2-amino-2-deoxy-D-glucopyranoside[8], in ethanol was boiled under reflux in the presence of dry Dowex 50 (H^+) resin for 16 h The products were *N*-deacetylated with 2M sodium hydroxide and assayed in the amino acid analyzer The α- and β-pyranosides were detected (30 1), but furanosides were not detected

The amino group of 2-amino 2-deoxy-α-D-glucopyranose is more basic (pK_a

1.95×10^{-8}) than that of the β anomer (pK_a 5.37×10^{-8})[9,10]. The phenyl group is involved in hydrophobic interaction with benzene rings of the resin matrix[11], as indicated by the R_{NH_2} values (1.93–4.86) of the phenyl 2-amino-2-deoxy-D-glycosides. These data indicate that the unique values of R_{NH_2} of the anomeric 2-amino-2-deoxy-D-glycosides are variously based on differences in the basicity of amino groups and in the hydrophobic interactions with the resin matrix.

EXPERIMENTAL

N-Deacetylation of 2-acetamido-2-deoxy-D-glycosides was performed[12] with 2M NaOH at 100° for 5 h.

The Hitachi KLA-2 amino acid analyzer used in the present work was equipped with a 50-cm column (0.9 × 48 cm) for resolution of methyl and ethyl 2-amino-2-deoxy-D-glycosides, and a 15-cm column (0.9 × 14 cm) for resolution of phenyl 2-amino-2-deoxy-D-glycosides. The columns were packed with Amberlite CG-120 resin (Type III) and eluted with 0.35M citrate buffer containing 0.2M boric acid (pH 5.09).

For preparative separations, a solution of a sample (0.5 g) of the mixture of 2-acetamido-2-deoxy-D-glycosides prepared by the Fischer[13] or Helferich[14] methods in 2M NaOH (10 ml) was kept at 100° for 3–5 h, then neutralized with 6M HCl, and diluted to ~150 ml with distilled water. An aliquot (~0.2 ml) was then assayed in the amino acid analyzer. The dilute solution was applied to a column (2.7 × 52 cm) of Amberlite CG-120(H^+) resin and eluted with 0.3M HCl. Fractions (7 ml) were collected, and examined by the ninhydrin and chromotropic acid reactions[15]. The fractions containing each pure anomer were combined, and concentrated *in vacuo*, with several additions of distilled water to afford the hydrochloride.

Analysis of authentic 2-amino-2-deoxy-D-glycosides by the amino acid analyzer was carried out as previously described[16].

REFERENCES

1 Preliminary report: S. Hirano, M. Ishigami, and Y. Ohe, *J. Biochem. (Tokyo)*, 78 (1975) 421–422.
2 Y. Matsushima, T. Miyazaki, and J. T. Park, *J. Biochem. (Tokyo)*, 54 (1963) 109–110.
3 Y. Matsushima and T. Miyazaki, *Bull. Chem. Soc. Jap.*, 38 (1965) 1325–1326.
4 A. Neuberger and B. M. Wilson, *Carbohydr. Res.*, 17 (1971) 89–95.
5 S. Beychok, G. Ashwell, and E. A. Kabat, *Carbohydr. Res.*, 17 (1971) 19–24.
6 P. W. Austin, F. H. Hardy, J. G. Buchanan, and J. Baddiley, *J. Chem. Soc.*, (1963) 5350–5353.
7 M. E. Evans, L. Long, Jr., and F. W. Parrish, *J. Chromatogr.*, 32 (1968) 602–605.
8 S. Hirano, Y. Ohe, and M. Ishigami, *J. Org. Chem.*, 41 (1976) 4038–4039.
9 A. Neuberger and A. P. Fletcher, *Carbohydr. Res.*, 17 (1971) 79–88.
10 A. Neuberger and A. P. Fletcher, *J. Chem. Soc. B*, (1969) 178–181.
11 J. Schneider and Y. C. Lee, *Carbohydr. Res.*, 30 (1973) 405–408.
12 M. Fujinaga and Y. Matsushima, *Bull. Chem. Soc. Jap.*, 37 (1964) 468–470.
13 G. N. Bollenback, *Methods Carbohydr. Chem.*, 2 (1963) 326–328.
14 J. Conchie and G. A. Levvy, *Methods Carbohydr. Chem.*, 2 (1963) 345–347.
15 D. J. Hanahan and J. N. Olley, *J. Biol. Chem.*, 231 (1958) 813–828.
16 S. Hirano, Y. Akimoto, M. Ishigami, and Y. Koga, *Anal. Biochem.*, 66 (1975) 627–628.