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SYNTHESIS OF THE ANOMERS OF METHYL 2-ACETAMIDO-2-DEOXY-D-GALACTO-FURANOSIDE AND -PYRANOSIDE*

MANJU SARKAR AND ELVIN A. KABAT

Departments of Microbiology, Human Genetics and Development, and Neurology, College of Physicians and Surgeons, Columbia University, New York, New York 10032 (U.S.A.)

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ABSTRACT

On treatment with methanol in the presence of Amberlite IR-120 (H^+) resin, 2-acetamido-2-deoxy-D-galactose yielded a mixture of four isomers, the methyl 2-acetamido-2-deoxy-α- and -β-D-galactofuranosides and their corresponding pyranosides. The isomers were separated preparatively on Dowex-1 ion-exchange resin, and analytically by high-pressure liquid chromatography, and identified by their m.p. and specific rotation and by assays of periodate uptake and formaldehyde liberated.

INTRODUCTION

Methyl glycosides of various 2-acetamido-2-deoxy sugars are of substantial importance in studying the combining sites of certain anticarbohydrate antibodies and of many lectins[1,2]. Beychok *et al.*[3] synthesized the four methyl glycosides of 2-acetamido-2-deoxy-D-mannose by refluxing it with methanol and Amberlite IR-120 (H^+) resin. Chromatography on Dowex-1 X2 (OH^-) resin[4] (200–400 mesh) using carbon dioxide-free water, gave two peaks, peak I (a mixture) and peak II, from which the methyl α-furanoside was obtained as a crystalline compound; three other methyl glycosides were separated by chromatography on Whatman No. 1 paper in 70:20:23 ethyl acetate–pyridine–water. Morgan and Neuberger[5] used this procedure in synthesizing the four methyl glycosides of 2-amino-2-deoxy-D-glucose. All four compounds were separated on Dowex-1 X2 (OH^-) resin, the elution being monitored by optical rotation at 365 nm; the compounds were then *N*-acetylated.

The present article reports the synthesis of the four methyl glycosides of 2-acetamido-2-deoxy-D-galactose (D-GalNAc) by the procedure of Beychok *et al.*[3]. Chromatography on Dowex-1 X2 (OH^-) resin[5] gave four peaks. The four isomers were also separated by high-pressure liquid chromatography (h.p.l.c.).

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RESULTS AND DISCUSSION

The four isomers of methyl 2-acetamido-2-deoxy-D-galactoside were prepared by treatment of D-GalNAc with methanol in the presence of Amberlite IR-120 (H^+) resin, and separated preparatively on an ion-exchange resin according to their relative acidities (see Fig. 1). These four isomers were also separated by using h.p.l.c.; this process was studied by changing the polarity of the solvent in order to obtain a good separation. Each isomer was tested for homogeneity by paper chromatography (p.c.), thin-layer chromatography (t.l.c.), and h.p.l.c. Paper chromatography (up to 300-μg samples) and t.l.c. (up to 50-μg samples) of each isomer gave a single spot, but, in h.p.l.c., the first fraction corresponding to the α-pyranoside was found to be a mixture of the α- and β-pyranosides; it was therefore passed through a column of freshly regenerated Dowex resin (see Fig. 2). The asymmetrical, main (and adjacent small) peaks were separated into four subfractions. The first subfraction was found to be homogeneous by h.p.l.c. and to consist of the α-pyranoside; the second was a mixture of the α- and β-pyranosides; and the third was mainly the β-pyranoside together with a very small proportion of the α-pyranoside. The fourth and fifth

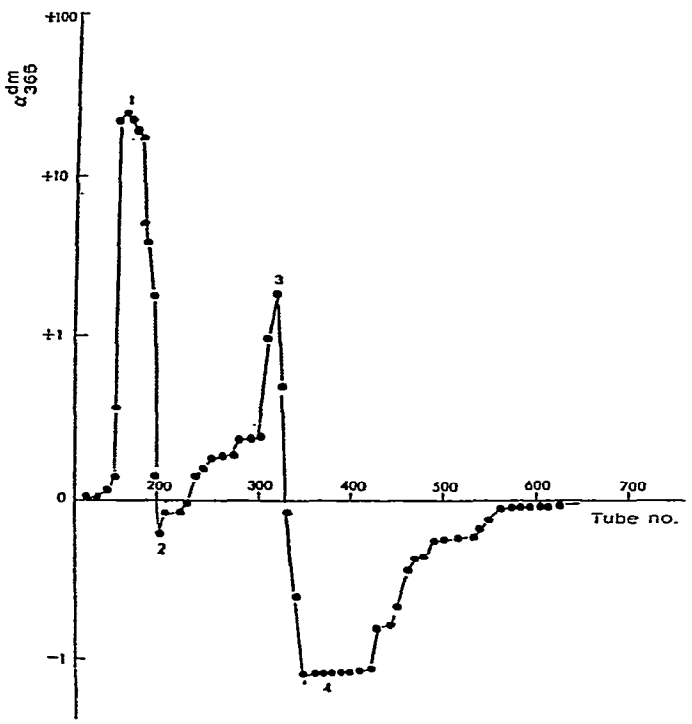


Fig. 1. Elution pattern of the four methyl 2-acetamido-2-deoxy-D-galactosides on Dowex 1-X2 (OH^-) resin. [Peak 1 (tubes 140–190), methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (2); peak 2 (tubes 195–225), methyl 2-acetamido-2-deoxy-β-D-galactopyranoside (3); peak 3 (tubes 305–320), methyl 2-acetamido-2-deoxy-α-D-galactofuranoside (4); and peak 4 (tubes 325–615), methyl 2-acetamido-2-deoxy-β-D-galactofuranoside (5).]

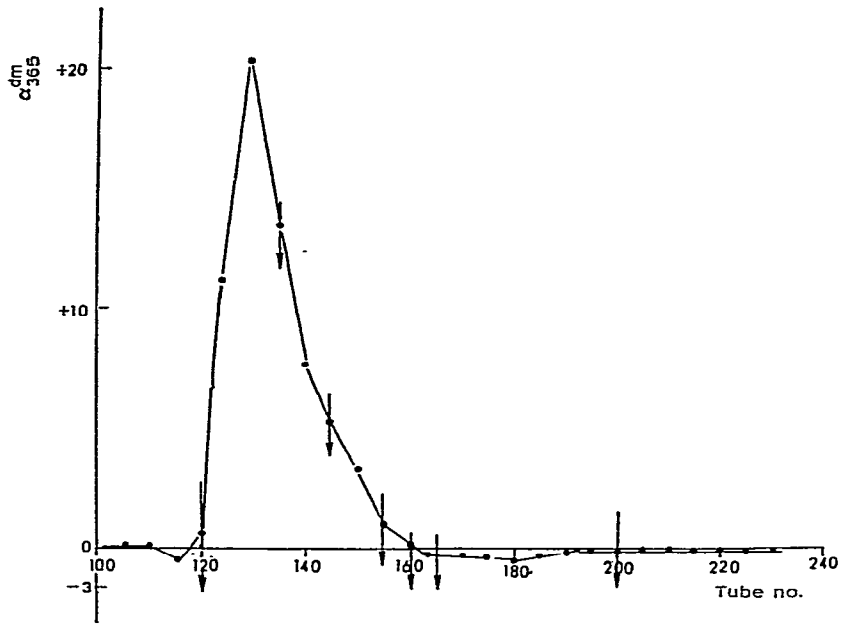


Fig. 2. Elution pattern of peak 1 repassed through a column of Dowex 1-X2(OH^-) resin. [The main peak was pooled into five fractions as shown.]

subfractions were β-pyranoside. In h.p.l.c., the β-pyranoside, α-furanoside, and β-furanoside each gave a single peak. Thus, it was very difficult to separate the α- and β-pyranosides in a single pass through a Dowex column. To obtain a good separation, the fractions obtained should be re-passed through a column of Dowex, and the most uniform fractions of the peaks selected.

The β-pyranoside separated by this procedure has a m.p. of 245°, higher than that previously reported[7] (190°). Three of the glycosides, the α-pyranoside, β-pyranoside, and β-furanoside, crystallized well, whereas the α-furanoside, originally a syrup, was obtained as a white solid from methanol–ether. The α and β anomers of the pyranosides and the furanosides were distinguished by their specific optical rotations at the D line of sodium, as well as by periodate uptake and formaldehyde produced. Total nitrogen as determined by the ninhydrin procedure[8] (with digestion with sulfuric acid) gave values close to the theoretical. Amino nitrogen, as determined by ninhydrin, but without digestion with sulfuric acid, was negligible, indicating that no deacetylation had occurred. Estimation of hexosamine tended to give low results, because *N*-deacetylation stabilizes the glycosidic linkage[9,10]; hydrolysis with 2M hydrochloric acid during 4 h gave the best results (see Fig. 3), values of 81 to 85% being obtained. *N*-Acetylhexosamine was determined after *N*-re-acetylation; D-GalNAc gives 31% of the color value of D-GlcNAc (used as the standard)[11,12].

In h.p.l.c., the original mixture of isomers showed two additional peaks. These were identified as the free hexosamine and one of its methyl glycosides, because, when the mixture was treated with Amberlite IR-120 (H^+) resin before h.p.l.c.,

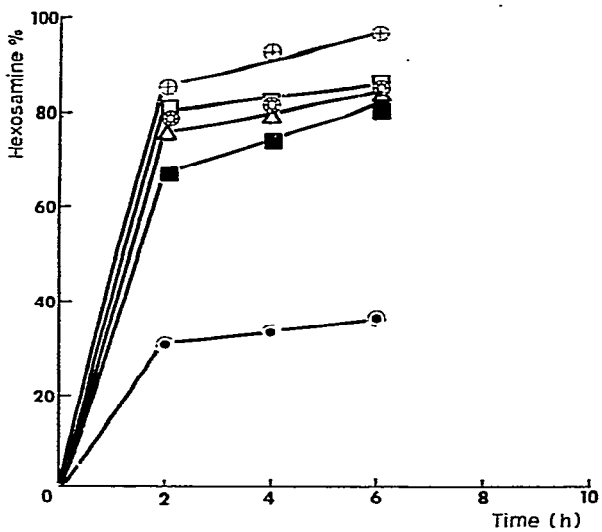


Fig. 3. Hexosamine liberated from the four isomers on hydrolysis for 2, 4, and 6 h in 2M HCl; [■ Methyl α-D-Gal*p*NAc; △ methyl β-D-Gal*p*NAc; □ methyl α-D-Gal*f*NAc; ◎ methyl β-D-Gal*f*NAc. ⊕ GalNAc; ● Cyst 9, a blood-group A substance which contains 32% of *N*-acetyl-hexosamine, used for comparison.]

the two peaks disappeared, and, on elution of the resin with acid, the two peaks reappeared, one being at the position of a methyl glycoside, and the other at the position of 2-amino-2-deoxygalactose; these were not found for the purified compounds.

EXPERIMENTAL

General. — Melting points were determined with a Fisher apparatus. Optical rotations were measured with a Model 141 Perkin–Elmer polarimeter, in a 1-dm cell holding 1 ml. Paper chromatography was conducted on Whatman No. 1 MM filter paper, and thin-layer chromatography, on plates of silica gel, the solvent system for both being 6:4:3 1-butanol–pyridine–water. Plates and papers were developed by spraying either directly with the alkaline silver reagent, or with this reagent following periodate oxidation. Reducing sugar was determined by means of alkaline ferricyanide[11]. H.p.l.c. was performed on a μBondapak-carbohydrate column (Waters Associates, Model No. 6000) with 19:1 acetonitrile–water at a flow rate of 0.6 to 0.8 ml/min.

Preparation of methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**2**), *methyl 2-acetamido-2-deoxy-α-D-galactofuranoside* (**4**), *methyl 2-acetamido-2-deoxy-β-D-galactopyranoside* (**3**), *and methyl 2-acetamido-2-deoxy-β-D-galactofuranoside* (**5**). — Amberlite IR-120 (H^+) ion-exchange resin was twice recycled between the hydrogen and sodium forms, successively washed thoroughly with water and methanol, and dried *in vacuo*. D-GalNAc (**1**) (1 g; Sigma Chemical Co.) was dried *in vacuo*, and

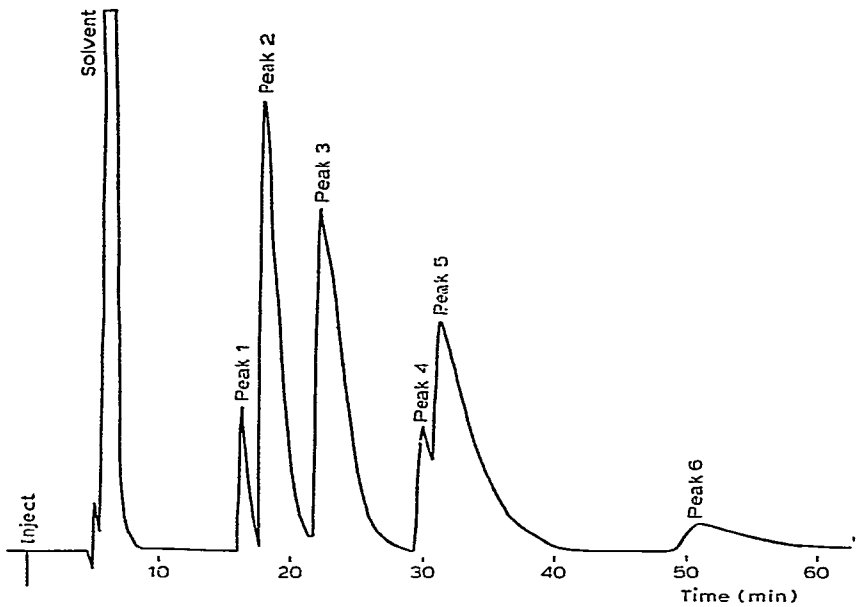
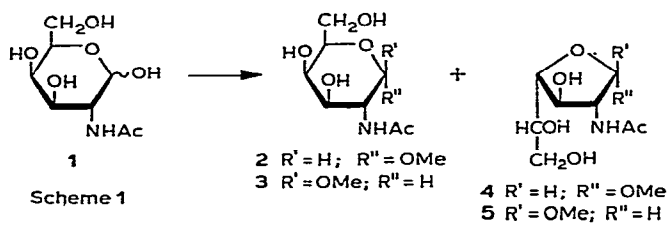


Fig. 4. Reaction mixture examined by h.p.l.c. [Peak 1, corresponds to α-furanoside; peak 2, to β-furanoside; peak 3, to α-pyranoside; peak 4 may be a methyl glycoside of 2-amino-2-deoxygalactose; peak 5 corresponds to β-pyranoside; and peak 6, to 2-amino-2-deoxygalactose.]

then dissolved in anhydrous methanol (25 ml), and dry Amberlite IR-120 (H^+) resin (500 mg) was added. The suspension was stirred magnetically and boiled under reflux with exclusion of moisture. Glycoside formation was monitored by measuring the decrease in reducing power of the solution[11]; after boiling for 45 min, no reducing sugar was present, and the reaction was stopped. A second portion (4 g) of D-GalNAc was treated in the same way. The resin was filtered off and washed several times with methanol, and the filtrates and washings were combined, and evaporated *in vacuo* to a pale-yellow syrup (4.27 g) which was examined by p.c. and t.l.c. No spots were revealed by the alkaline silver reagent, but, on spraying with periodic acid followed by the alkaline silver reagent, two spots were seen on the paper chromatogram, and four on the t.l. chromatogram. A sample of the syrup was examined by h.p.l.c. on a μBondapak-carbohydrate column using 19:1 acetonitrile–water. Four main and two minor peaks were obtained (see Fig. 4).



Scheme 1

Scheme 1. Structures of D-GalNAc (**1**), methyl α-D-Gal*p*NAc (**2**), methyl β-D-Gal*p*NAc (**3**), methyl α-D-Gal*f*NAc (**4**), and methyl β-D-Gal*f*NAc (**5**).

To separate the glycosides, a solution of the syrup (3.8 g) in CO_2-free, distilled water (6 ml) was applied to a column (102 × 3.5 cm) of Dowex-1 X2 (OH^-) ion-exchange resin (200–400 mesh) that had been recycled twice between the hydrogen and hydroxide forms. The column was eluted with CO_2-free, distilled water, samples (1.2 ml) being collected, and their optical rotation at 365 nm was measured (see Fig. 1). Fractions containing the separated isomers were combined as shown, and evaporated at 40° under diminished pressure.

Fraction I (2.3 g) afforded methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**2**); it gave one spot in p.c. and t.l.c., but two peaks in h.p.l.c. It was re-passed through a Dowex column (see Fig. 2); 5 subfractions were collected, and each was examined by h.p.l.c. The first (423 mg), compound **2**, gave a single peak in h.p.l.c.; the second (341 mg) was a mixture of α-pyranoside and β-pyranoside; the third (369 mg) was mainly β-pyranoside, with a small proportion of α-pyranoside; and fourth (16.7 mg) and fifth subfractions (16.3 mg) were essentially β-pyranoside. The first three subfractions crystallized (and, on recrystallization from absolute alcohol, the third subfraction yielded 127 and 27 mg of **3**, in two crops).

The α-pyranoside was thrice recrystallized from ethanol, m.p. 218° (lit.[5] m.p. 217–218°), $[\alpha]_D^{24}$ +169° (*c* 0.1, water) (lit.[5] $[\alpha]_D$ +170°). Upon treatment with sodium periodate, 1.05 moles of periodate were consumed per mole of sample, with no liberation of formaldehyde.

Anal. Found: total N, 6.3% (Calc.: 6.0%); amino N, 0.035% (Calc.: 0%); free hexosamine[11], 0% (Calc.: 0%); hexosamine (after hydrolysis for 6 h in 2M HCl), 81% (Calc.: 100%); *N*-acetylhexosamine[11] (hydrolysis for 6 h), 31.2% (Calc.: 31%).

Fraction II (245 mg) consisted of compound **3**; it gave one spot in p.c. and t.l.c., and one peak in h.p.l.c., and crystallized from absolute alcohol, m.p. 230–235° (lit.[7] m.p. 190°), $[\alpha]_D^{24}$ −4° (*c* 0.11, water) (lit.[7] $[\alpha]_D$ −12°). One mole of fraction II consumed 1.2 moles of periodate, liberating no formaldehyde.

Anal. Found: total N, 6.3% (Calc.: 6.0%); amino N, 0.021% (Calc.: 0%); free hexosamine, 1.8% (Calc.: 0%); hexosamine (after hydrolysis for 6 h with 2M HCl), 84% (Calc.: 100%); *N*-acetylhexosamine (after hydrolysis for 6 h with 2M HCl), 34% (Calc.: 31%).

Fraction III (143 mg) consisted of compound **4**, and was a syrup. A white, amorphous solid was obtained on attempting to crystallize it from methanol–ether; it had $[\alpha]_D^{24}$ +19° (*c* 0.3, water). In p.c., 300 μg, and in t.l.c. 50 μg, gave a single spot, and h.p.l.c. gave a single peak. One mole of fraction III consumed 1.1 moles of periodate, liberating 1.0 mole of formaldehyde.

Anal. Found: total N 6.1% (Calc.: 6.0%); amino N, 0.14% (Calc.: 0%); free hexosamine, 0.61% (Calc.: 0%); hexosamine (after hydrolysis for 6 h) 86% (Calc.: 100%); *N*-acetylhexosamine (after hydrolysis for 6 h) 31% (Calc.: 31%).

Fraction IV (692 mg) consisted of compound **5**, and it crystallized from absolute alcohol. It gave a single spot in p.c. and in t.l.c., and a single peak in h.p.l.c.: m.p. 168–170°, $[\alpha]_D^{24}$ −72.6° (*c* 0.12, water). It consumed 1.1 moles of periodate per mole of sample, liberating 1.1 moles of formaldehyde.

Anal. Found: total N, 6.2% (Calc.: 6.0%); amino N, 0.08% (Calc.: 0%); free hexosamine, 1.3% (Calc.: 0%); hexosamine (after hydrolysis for 6 h with 2M HCl) 85% (Calc.: 100%); *N*-acetylhexosamine, 33% (Calc.: 31%) relative to D-GlcNAc.

Separation of anomers by h.p.l.c. — The reaction mixture (1.25 mg) was injected into a column of μBondapak–carbohydrate. Six peaks were obtained, instead of four (see Fig. 4). Each purified, and chemically identified, isomer (~1.2 mg) was injected separately. Peak 1 corresponded to the α-furanoside, peak 2 to the β-furanoside, peak 3 to the α-pyranoside, and peak 5 to the β-pyranoside. Peaks 4 and 6 were identified as follows. The reaction mixture (15 mg) was treated with Amberlite IR-120 (H^+) resin, and the solution was evaporated, and the residue dried (yield, 12.5 mg). This sample (~1.5 mg) gave four peaks, corresponding to h.p.l.c. peaks 1, 2, 3, and 5, and peaks 4 and 6 had disappeared. The resin was eluted with 2M HCl, the solution evaporated, and the residue dried *in vacuo*, and then examined by h.p.l.c.; the two missing peaks were now seen, and were collected separately and tested by t.l.c. on two plates. When one plate was sprayed with the alkaline silver reagent, peak 6 gave one spot, corresponding to 2-amino-2-deoxygalactose. The other plate was sprayed with periodate followed by the alkaline silver reagent, and gave single spots for the compounds in peaks 4 and 6.

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