Biochemical Studies on Carbohydrates

CLXXXVII. Oligosaccharides Separated after Acetolysis of the Group Mucopolysaccharide from Pig Gastric Mucus

Second Report: Acetylglucosamine-4- β -galactoside (Acetyllactosamine)

By

Hajime Masamune and Zensaku Yosizawa

(正宗 一)

(吉沢 善作)

From the Medico-chemical Institute, Tohoku University, Sendai

(Received for publication, October 30, 1955)

In the foregoing work¹⁾, the group mucopolysaccharide from pig stomach mucus was acetolyzed, followed by deacetylation and fractionation by means of a cellulose powder column, and gastro-N-trisaccharide was isolated from Frs. 49-90 of the 90 fractions. The present account deals with acetylglucosamine-4- β -galactoside which has been separated in crystalline form from the 41st-48th fractions. It has $[\alpha]_D^{25}$ in water of 28.8° at equilibrium and melts at $169-171^\circ\text{C}$. It proved to be composed of equimolecular acetylglucosamine and galactose and gave phenyllactosazone. Yosizawa,²⁾ one of the writers, separated formerly this disaccharide from a similar material, though amorphous and not perfectly pure, and established its structure. Later on Tomarelli *et al.*³⁾ and Zilliken *et al.*⁴⁾ isolated it from commercial hog gastric mucin, and Kuhn and Kirschenlohr⁵⁾⁶⁾ from the blood group substance of meconium. The present substance showed the infrared spectrum identical with that of the synthetical product of Kuhn and Kirschenlohr.⁶⁾

EXPERIMENTAL

Isolation of acetylglucosamine-4- β -galactoside

The 41st-48th of the 90 fractions of the deacetylated acetolysis products (starting material 20 g.) showed spots of galactose and two sugars of lower R_f values, when chromatographed with butanol-pyridine-water (5:3:2 by volume) according to Masamune and Yosizawa.⁷⁾ Those fractions were distilled together to syrupy consistency and redistilled with 30 cc. portions of abs. methanol, and the final dried residue was boiled with three 50 cc. portions of abs. methanol in succession (Each of the solutions was separated by centrifugation while warm). The solutions were united and distilled to 2-3 cc. (A few drops of octylalcohol were

used to evade foam formation), when crystals began to appear. The condensate was placed in an ice-chamber overnight. The deposit was collected on a suction funnel and washed with 3 cc. of ice-chilled abs. methanol, 5 cc. of abs. ethanol and 5 cc. of dry ether in turn. 0.4 g. was the yield here. The crude substance was recrystallized from abs. methanol three times in a similar manner. The product had the crystalline form in Fig. 1, and melted at 169–171°C on heating. Molisch positive, Morgan-Elson negative.

Properties of the disaccharide

1) When chromatographed with solvent butanol-pyridine-water (5:3:2 by volume) according to Masamune and Yosizawa⁷⁾ (Sliver Toyo Roshi filter paper no. 3), it moved with an average R_r (rate of flow as compared with rhamnose) value of 0.46 and an average R_g (rate of flow as compared with glucose) value of 0.76. The spot was colored orange-brown by the aniline hydrogen phthalate reagent of Partridge.

Fig. 1. Microphotograph of crystals of the disaccharide.

2) *Optical rotation.* 10.4 mg. of the substance were dissolved in 1 cc. of water at 18°C and examined as for its rotation in a 1 dm. tube.

Time after dissolution (min.)	α (°)	$[\alpha]_D^{18} = \frac{\alpha \times 100}{1 \times 1.04}$ (°)
5	+0.50	+48.1
10	+0.48	+46.2
20	+0.44	+42.3
30	+0.42	+40.4
60	+0.37	+35.6
2 × 60	+0.32	+30.8
3 × 60	+0.30	+28.8
24 × 60	+0.30	+28.8

By extrapolation to time zero is obtained $[\alpha]_D^{18}$ of +51.5° as the initial rotation. The half value time of mutarotation is 32 minutes.

3) *Paper partition chromatography of the sugar components.* 10 mg. of the substance were hydrolyzed with 2 cc. of 0.5 *N* HCl in a sealed tube by heating at 100°C for 10 hours. The hydrolysate was transferred to a small dish and evaporated at 30–40°C in vacuo over solid CaCl_2 and NaOH. The residue was subjected repeatedly to dissolution in water and evaporation in order to expel off HCl as far as possible. The final dried residue was taken up in 0.2 cc. of water, and a 0.02 cc. portion of the solution was sampled on a paper sliver to chromatograph as described above. Glucosamine and galactose were identified (Fig. 2).

Fig. 2. Butanol-pyridine-water (5:3:2 by volume) -chromatogram of the hydrolysate of the disaccharide. Sliver 12×55 cm. Irrigation descending. Temperature and duration of test 24–26°C and 16 hrs. respectively. Indicator aniline hydrogen phthalate. The standard solution was 1% with respect to individual sugars hexosamine hydrochlorides and galactose.

a : reference run. b : main run. 1 : galactosamine. 2 : glucosamine. 3 : galactose.

4) *Quantitative analysis.* See Table I. The molecular weight calculated from the iodine use amounts to 423.

TABLE I
Composition of the Disaccharide

Figures express percentages.

Analysis	Found	Calcd. for $C_{14}H_{25}O_{11}N \cdot CH_3OH$ (415.4)
N*	3.33	3.37
C†	43.27	43.37
H†	7.01	7.03
Glucosamine‡	42.8	43.1
Galactose§	42.0	43.3
Acetyl	10.1	10.4
Iodine use as glucose¶	42.6	43.3

* Micro Kjeldahl. † Pregl; Kindly analysed by Mr. M. Suzuki in the Chemical Department. ‡ Masamune and Yosizawa.⁸⁾ § Masamune and Sakamoto.⁹⁾ || Suzuki.¹⁰⁾ ¶ Macleod and Robison.¹¹⁾

5) *Infrared spectrum* (The writers are indebted to Mr. Y. Ikegami in the Chemical Institute of Non-aqueous Solutions). The infrared absorptions were measured in Perkin-Elmer Model 21 recording infrared

spectrophotometer with a NaCl prism by the KBr disk method. The spectrum obtained is shown in Fig. 3, which coincides perfectly with that of 2-desoxy-2-acetamino-lactose synthesized by Kuhn and Kirschenlohr.⁶⁾

Fig. 3. Infrared spectrum of the disaccharide.

6) *Phenylosazone*. The procedure of Kuhn & Kirschenlohr⁵⁾ was applied. 100 mg. of the substance, 1 g. of phenylhydrazine hydrochloride and 0.5 g. of crystalline sodium acetate were dissolved together in 7 cc. of water, and heated for 1.5 hours in a boiling water-bath. The oily osazone was kneaded with water, and the centrifugate was taken up in 2-3 cc. of hot water and stood in air. 9 mg. of crystals appeared as yellow red needles. They were recrystallized three times. F.P. 203-205°C. N. (Friedrich's micro Kjeldahl method) 10.48% (Calcd. for $C_{24}H_{32}N_4$: 10.76%). Mixtures of the product with phenyllactosazone prepared with lactose showed no depression of melting point.

The expenses of this work were defrayed by a grant from the Education Department through the Grant Committee for Scientific Researches. H. Masamune.

References

- 1) Masamune, Yosizawa & Haga, *Tohoku J. Exp. Med.*, this volume, p. 257.
- 2) Yosizawa, *ibid.*, 1949, **51**, 51; 1950, **52**, 111, 145.
- 3) Tomarelli, Hassinen, Eckhardt, Clark & Bernhart, *Arch. Bioch. & Biophys.*, 1954, **48**, 225.
- 4) Zilliken, Smith, Tomarelli & György, *ibid.*, 1955, **54**, 398; Zilliken, Smith, Rose & György, *J. Biol. Chem.*, 1954, **208**, 299.
- 5) Kuhn & Kirschenlohr, *Chem. Ber.*, 1954, **87**, 560.
- 6) Kuhn & Kirschenlohr, *ibid.*, 1954, **87**, 1547.
- 7) Masamune & Yosizawa, *Tohoku J. Exp. Med.*, 1953, **59**, 1.
- 8) Masamune & Yosizawa, *ibid.*, this volume, p. 53.
- 9) Masamune & Sakamoto, *ibid.*, 1956, **63**, 345.
- 10) Suzuki, *J. Bioch. (Japan)*, 1938, **27**, 367.
- 11) Macleod & Robison, *Bioch. J.*, 1929, **23**, 517.