Biochemical Studies on Carbohydrates

CCXVI. On Conditions of Acetolyzing Molisch-positive Mucopolysaccharides

By

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Since Franchimont¹⁾ applied acetolysis to cellulose in 1879, the conditions for fission in that way of cellulose²⁾ but not of Molisch-positive mucopolysaccharides have been investigated scrupulously. In the structural study of the group mucopolysaccharide from pig stomach mucus,^{3), 4)} we applied the procedure, which Bergmann *et al.*⁵⁾ had used for acetolysis of chitin, and its minor modifications. However, oligosaccharide acetates were thereby yielded little as compared with monosaccharide acetates, and besides, a large amount of the material remained unattacked. Hence we have investigated how to raise the yield of oligosaccharides particularly disaccharides by the experimental scheme following: 1) acetylation of a Molisch-positive mucopolysaccharide in order to change it soluble in the acetolysis reagent, 2) standing portions of the mucopolysaccharide acetate with a glacial acetic acid-acetic anhydride mixture (1:1 by volume) containing a various amount of H_2SO_4 at different temperatures for different durations of time, 3) deacetylation of the products, and 4) column chromatography of the deacetylation products to compare the yields of various fractions monosaccharides, disaccharides, oligosacchrides excepting disaccharides, and polysaccharides with one another. The results indicated that the best yield of disaccharides is obtained when acetolysis is effected with the glacial acetic acid-acetic anhydride mixture containing 4% H_2SO_4 at 30°C for 4 days and that of oligosaccharides exclusive of disaccharides is given when the H_2SO_4 concentration is increased to 8% and acetolysis is continued for 15 days at 20°C. Under the latter conditions the disaccharide fraction is produced in quite good yield simultaneously.

EXPERIMENTAL

Material and Method

- 1) Material. The group mucopolysaccharide from pig stomach

mucus which had been prepared according to Sinohara,⁴⁾ was used as the material.

2) Method. a) *Preliminary acetylation of the mucopolysaccharide.* The procedure of Carson and Macley⁶⁾ was adapted as follows. 10 g. of the mucopolysaccharide was taken up in 50 cc. of formamide and shaken at room temperature for 30 minutes, whereby it dissolved clearly. To the solution, 100 cc. of dry pyridine was added slowly at room temperature at first and afterwards 90 cc. of acetic anhydride was added in small portions during 20 minutes under cooling on ice. The mixture was shaken at room temperature for 1 hour and then stood at 30°C for 2 days with occasional shaking. The reaction mixture tinged yellowish was poured into 3 l. of 2% HCl containing about 500 g. of smashed ice, stirred for 1 hour and centrifuged. The centrifugate was washed with 100 cc. of ice-chilled 0.5% HCl and two 100 cc. portions of ice-chilled water, suspended in 100 cc. of water and placed in a refrigerator. Centrifuged. The centrifugate here was dried at 35°C under reduced pressure over solid caustic soda and anhydrous calcium chloride. 12.1 g. of a white powder was obtained.

It had $[\alpha]_D^{20}$ of $\frac{-0.503 \times 100}{1.65 \times 1} = -30.5^\circ$ in a mixture of methanol and chloroform in the volume proportion of 1:1. N (micro Kjeldahl) 3.5%, acetyl (Suzuki⁷⁾) 33.0%, hexosamine (Masamune and Yosizawa⁸⁾) 28.1%, galactose (Reaction HR₁₀ of Masamune and Sakamoto⁹⁾) 19.9%, L-fucose (a modification of Dische and Shettles¹⁰⁾) 9.8%, sialic acid as C₁₁H₁₉NO₁₀ (Werner and Odin's direct Ehrlich reaction¹¹⁾) 0.9%, ash (Pregl method without use of H₂SO₄) 0%.

b) *Acetolysis of the acetylated mucopolysaccharide.* A typical example will be described. 9 g. of the mucopolysaccharide acetate was taken up in 90 cc. of a mixture of equal volumes of glacial acetic acid and acetic anhydride and shaken at room temperature for 30 minutes until it dissolved transparently. The solution was chilled on a freezing mixture (about -5°C), 4 cc. of concentrated sulfuric acid was added drop by drop under agitation, and the mixture was stood at room temperature for 2 hours. Turbidity that had occurred on the addition of sulfuric acid disappeared. The solution was further stood in a thermostat at 30°C with occasional shaking. When 4 days had elapsed, a 30 cc. portion of the solution was poured into a mixture of 1 l. of 60% (v/v) methanol containing 150 g. of smashed ice and 300 g. of Amberlite IR 45 (OH form) under vigorous agitation, agitated continuously for 30 minutes and further stood at room temperature for 6 hours. Filtered. The resin on the funnel was washed with three 100 cc. portions of methanol. The filtrate and washings were united and distilled in vacuo to about 100 cc. To the condensate was added 100 cc. of methanol, with following distillation to

100 cc. under reduced pressure. This operation was repeated several times to expel the acetic acid, and then the solution was distilled to dryness and placed in a desiccator. 3 g. of a yellowish brown powder was given. From the balance of the reaction mixture above, 30 cc. portions were taken out at 4 days' intervals to treat similar to above. The yields of the acetolysis products here were also 3 g.

c) *Deacetylation.* It was conducted according to Kuhn and Kirschenlohr.¹²⁾ 3 g. of an acetolysis product was dissolved in 60 cc. of absolute methanol, 60 cc. of methanol saturated with NH_3 at 0°C was added, and the mixture was placed in a thermostat at 20°C for 6 hours with subsequent distillation in vacuo at below 20°C . The still-residue was dissolved in 20 cc. of methanol and again distilled. The residue thus given was taken up in 15 cc. of water, the insoluble part was rejected, and the solution was added to 1.5×3 cm. columns of Dowex 50 (H form) and of Amberlite IR 45 (OH form) in turn and washed with 50 cc. of water. The effluent and washing were distilled in vacuo to dryness and placed in a desiccator. About 1.3 g. of a yellowish brown powder was yielded from each of the acetolysis products.

d) *Column chromatography of the deacetylated products.* The procedure of Whistler and Durso¹³⁾ was adapted, employing a column packed of charcoal alone. Namely, 1.3 g. of a deacetylated acetolysis product was dissolved in 10 cc. of water, added to a 3×10 cm. column and eluted by the aid of two 1 l. portions of water, 1.5 l. each of 2.5% (v/v), 5% and 7.5% ethanol and 2 l. each of 10%, 20%, 30% and 50% ethanol and 50% acetone in succession. The consecutive effluents were separately distilled under reduced pressure to dryness. All the still-residues were subjected to determination of iodine use (See e)), and those of the first two eluates to paper chromatography of sugar components in addition.

e) *Determination of iodine use.* The method of Whistler and Tu¹⁴⁾ was modified as follows. 20–30 mg. substance was dissolved in 1 cc. of water and bufferized with 3 cc. of the Na_2HPO_4 -NaOH buffer of pH 11.2 and I 0.54, and immediately thereupon 2 cc. of 0.05 N I_2 was added. The mixture was hermetically stoppered, stood in a water-bath at 20°C for exactly 30 minutes avoiding light, acidified with 1 cc. of 1 N H_2SO_4 and titrated with 0.01 N $\text{Na}_2\text{S}_2\text{O}_3$. The control run with glucose and the blank were carried out in parallel.

Results with Comment

1) The still-residue of the first effluent in d) of the second section showed spots of L-fucose, N-acetylhexosamine and galactose in a paper chromatogram developed by descending irrigation with Solvent butanol-

TABLE I
Yields and Analyses of Various Fractions of the Deacetylated Products, when the Mucopolysaccharide Acetate Was Acetolyzed under Various Conditions

Combined conditions of acetolysis																		Analysis		
No.		Yield in per cent of total sum														Iodine use as glucose (%)		Ash* (%)		
Fr.	Name	Volume (l.)															91.0	0.5		
A	Water	1.0	8.0	22.2	27.2	13.0	27.0	9.5	23.5	33.2	25.8	30.5	38.2	54.0	65.0	33.4	42.0	37.2	54.5	0.0
	Water	1.0	3.0	3.0	4.3	5.0	5.8	2.8	4.2	2.2	6.0	6.1	4.8	3.0	3.0	4.0	3.7	5.5	45.6	0.0
	2.5% (v/v) ethanol	1.5	4.9	4.5	8.8	9.3	7.4	4.0	8.1	10.5	10.0	10.0	10.1	6.2	5.0	11.3	9.3	8.2	41.9	0.0
	5% (v/v) ethanol	1.5	3.8	5.2	4.5	8.8	5.9	4.2	10.1	6.4	5.4	4.0	3.2	4.3	4.1	6.6	5.9	6.5	40.8	0.0
	7.5% (v/v) ethanol	1.5	3.4	5.1	5.0	6.9	5.7	4.8	9.3	4.6	5.9	5.2	4.2	4.2	4.1	6.4	4.7	6.3	36.0	0.0
C	10% (v/v) ethanol	2.0	5.5	5.6	2.4	7.8	8.9	4.8	7.5	3.7	5.2	5.3	5.6	4.8	2.9	5.9	5.2	5.9	31.9	0.0
	20% (v/v) ethanol	2.0	5.2	14.2	12.8	7.4	10.3	8.1	7.0	12.4	10.8	8.3	6.9	3.7	3.0	5.9	7.5	7.1	21.4	0.0
	30% (v/v) ethanol	2.0	4.5	4.5	2.5	3.0	5.8	7.0	3.5	4.9	4.6	3.1	3.3	2.6	2.3	4.0	6.6	4.2	16.0	0.0
	50% (v/v) ethanol	2.0	6.8	6.6	9.5	6.8	5.5	10.2	3.5	1.2	1.2	1.0	2.2	3.7	2.7	3.2	2.2	3.2	15.3	0.0
	50% (v/v) acetone	2.0	7.8	7.4	6.2	8.0	5.2	5.5	1.7	4.3	1.1	2.8	1.9	1.6	3.0	1.5	1.6	4.6	18.8	11.3
D			47.1	27.1	16.8	24.0	12.5	39.1	21.6	17.6	24.0	23.7	19.6	11.9	4.9	18.8	11.3	11.3

Fig. 1. Yields of Fr. A, B, C and D in per cent of the respective deacetylated products when the mucopolysaccharide acetate was acetolyzed under combined conditions Nos. I—V (temp. 20°C) (a), Nos. VI—XIII (temp. 30°C) (b) and Nos. XIV—XVI (temp. 40°C) (c).

pyridine-water (5:3:2)¹⁵⁾ (Toyo Roshi filter paper No. 3; 25°C; spray reagent the aniline hydrogen phthalate reagent of Partridge), and that of the second effluent gave a spot with R_{lactose} of 1.33 in a paper chromatogram developed in a similar manner.

2) Yields of the fractions are embodied together with their iodine uses in Table I and graphically expressed in Figs. 1a—1c. The fractions reducing iodine as much as 90, 55–40 or 40–15% as glucose and that which was not eluted with 50% acetone are designated Fr. A, B, C and D respectively. The amount of the last fraction was calculated by subtracting the sum of Fr. A, B and C from the amount taken for the chromatographic fractionation.

Acetolysis at 20°C. 2% H_2SO_4 is too weak for sufficient acetolysis. Fr. C predominated in yield when 4% and 8% H_2SO_4 were employed.

The use of 8% H_2SO_4 at 20°C for 15 days is most suited to obtain Fr. C. Thereby Fr. B is yielded in a quite large amount though not so large as is given under combination number VII of conditions (See Table I), so that those conditions are recommendable for separating Fr. B as well as Fr. C at the same time.

Acetolysis at 30°C. The yields of Fr. A and B were small, when 2% H_2SO_4 was used. On the contrary, a great part of the material was broken up into monosaccharides by 12% or 16% H_2SO_4 . 4–8% are appropriate concentrations of H_2SO_4 to obtain Fr. B. In this case, the yield of Fr. A is raised a little by prolongation of the duration of acetolysis at the expense of Fr. B but those of Fr. C and D are not.

Fr. B is yielded most abundantly, when acetolysis is effected with 4% H_2SO_4 at 30°C for 4 days.

Acetolysis at 40°C. Most of the material was disintegrated into Fr. A by 4 and 8% H_2SO_4 , the other fractions particularly Fr. D being decreased. 40°C is therefore not adequate for effective acetolysis to obtain Fr. B or C.

Duration of acetolysis. It has no marked influence upon the relative amounts of the fractions given, suggesting that equilibrium takes place when acetolysis has proceeded to a certain extent.

SUMMARY

The group mucopolysaccharide from pig stomach mucus was acetylyzed with reagents containing various amounts of H_2SO_4 for various durations at various temperatures to determine the best conditions for obtaining disaccharides and oligosaccharides excepting disaccharides.

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