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Preliminary communication

A new method for the *N*-deacetylation of carbohydrates

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Amino sugars, generally 2-amino-2-deoxy sugars, are common constituents of polysaccharides and are usually *N*-acetylated. After *N*-deacetylation, these sugar residues offer starting points for specific degradations[1], either by acid hydrolysis, when the 2-amino-2-deoxyglycosidic linkages are resistant, or by deamination with nitrous acid, when the amino sugar residues are modified in various ways, depending upon their configuration[2]. *N*-Deacetylation may be achieved by treatment with sodium hydroxide or with hydrazine. It has proved difficult, however, to obtain quantitative *N*-deacetylation without simultaneous degradation. Anhydrous hydrazine, in the presence of hydrazine sulfate, a reagent introduced by Yosizawa *et al.*[3], has been used by Kochetkov and coworkers, both in model experiments[4,5] and in studies of bacterial polysaccharides[6,7]. In our hands, however, and also for one of the polysaccharides referred to above[7], this reagent has not been entirely successful, especially for polysaccharides in which the 2-acetamido-2-deoxy-D-glucopyranosyl residues are substituted at O-3. Two such polysaccharides, prepared from the cell-wall lipopolysaccharides of *Escherichia coli* O:69[8] and *Shigella flexneri*[9] by mild hydrolysis with acid, were only partially *N*-deacetylated on treatment with hydrazine–hydrazine sulfate at 105° for two days, and prolonged reaction resulted in extensive degradation.

We now report a new method for *N*-deacetylation, involving treatment with sodium hydroxide in aqueous methyl sulfoxide at ~100°. Sodium thiophenolate was added as an oxygen scavenger. It also seemed to have a catalytic effect.

In a series of reactions, the polysaccharide (10 mg) from *E. coli* or *S. flexneri* (referred to above) and sodium thiophenolate (100 mg) were dissolved in water (1 ml), 2M sodium methylsulfinylmethanide in methyl sulfoxide (5 ml) was added, and the mixture was heated at 80 or 100° in a sealed tube. Some sodium hydroxide was precipitated when the reagents were mixed, and a precipitate, consisting of diphenyl disulfide, was formed during the reaction. The reaction mixture was diluted with water, filtered, dialysed against water, and lyophilized. The recovery of polysaccharide was almost quantitative. The degree of *N*-deacetylation was followed by comparing the intensities of the *N*-acetyl signal at δ 2.06 and another typical signal, *e.g.*, for the methyl protons in a 6-deoxyhexose residue, in the 100-MHz ^{1}H-n.m.r. spectrum. In some experiments, the sodium thiophenolate was omitted.

TABLE I

N-DEACETYLATION OF POLYSACCHARIDES FROM *E. coli* O:69 AND *S. flexneri* UNDER CONDITIONS GIVEN IN THE TEXT

Polysaccharide	*Reaction temp. (degrees)*	*Reaction time (h)*	*Sodium thiophenolate*	*N-Deacetylation (%)*
E. coli	100	15	+	100
E. coli	100	10	+	96
E. coli	100	10	−	85
E. coli	80	48	+	96
E. coli	80	22	+	88
E. coli	80	22	−	82
S. flexneri	100	15	+	100
S. flexneri	80	7	−	96
S. flexneri	80	5	−	88
S. flexneri	80	2	+	80
S. flexneri	80	2	−	70
S. flexneri	80	0.5	+	40
S. flexneri	80	0.5	−	34

The results are summarized in Table I.

The molecular weight of the *E. coli* polysaccharide was determined before and after *N*-deacetylation, using gel filtration on a column containing equal amounts of Sephadex G-100 and G-200 calibrated with dextran fractions of known molecular weights[10]. The molecular weight decreased from M_w = 20,200, M_n = 5900 for the original polysaccharide to M_w = 11,300, M_n = 3400 for a product that had been *N*-deacetylated by treatment as above for 15 h at 100°. Somewhat lower values (M_w = 10,900 and M_n = 3000) were obtained for a sample treated for 10 h at 100°, but without sodium thiophenolate.

The main effect of sodium thiophenolate is in trapping any oxygen present and thus restricting the degradation of the polysaccharide. The degree of *N*-deacetylation under otherwise comparable conditions is, however, consistently higher in the presence of sodium thiophenolate, which consequently also exerts some catalytic effect. It is also evident from Table I that *N*-deacetylation of the *S. flexneri* polysaccharide is much faster than of the *E. coli* O:69 polysaccharide.

The method was also tested on methyl 2-acetamido-2-deoxy-α-D-glucopyranoside. A mixture of this glycoside (100 mg), sodium thiophenolate (40 mg), water (0.5 ml), and 2M sodium methylsulfinylmethanide in methyl sulfoxide (2.5 ml) was heated in a sealed tube at 100° for 4 h. The product was diluted with water (10 ml), neutralized with acetic acid, and filtered through a column (25 × 1 cm) of Dowex 50 (H^+) resin. The column was washed first with water (100 ml) and then with 0.5M hydrochloric acid (200 ml). On concentration of the latter eluate, an almost quantitative yield of methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride was obtained.

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