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Research Note

Pyridinium Substitution during the Sulfation of Polysaccharides in the Presence of Pyridine

ABSTRACT

Sulfation of polysaccharides with the chlorosulphonic acid/pyridine reagent leads to the incorporation of pyridinium substituents on a proportion of the reducing end-groups. The effect becomes more noticeable at lower molecular weights. N-α-D-xylopyranosyl pyridinium bromide was thus isolated from a hydrolysate of a sulfated xylan.

INTRODUCTION

Sulfated polysaccharides exhibit a wide range of pharmacological properties with particular interest being focussed in recent times on their inhibition of HIV-1 and other viruses (Baba *et al.*, 1988).

One of the most commonly used procedures for sulfating polysaccharides employs a mixture of chlorosulfonic acid and pyridine, the latter functioning as base and solvent (Ohle, 1922; Grönwall *et al.*, 1945).

We have recently found that under these conditions, pyridinium substituents may be incorporated into the products and their presence demonstrated by UV and NMR. The problem becomes increasingly severe at lower molecular weights. In this communication, we present evidence for the nature of the linkage.

EXPERIMENTAL

^{1}H-NMR spectra were recorded in D_2O at 199·5 MHz on a Jeol FX 200 using sodium 3-trimethylsilyl tetradeuteropropionate as internal standard. Beech xylan was a gift from Professor B. Lindberg, Stockholm University.

N-α- and -β-D-xylanpyranosyl-pyridinium bromide (1 and 2, respectively) were prepared essentially as described earlier (Hosie *et al.*, 1984).

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Sulfation of beech xylan

Chlorosulfonic acid (30 ml) was added slowly to pyridine (100 ml) with stirring and cooling.

Beech xylan (20 g) was then added and the reaction stirred at 70–75°C for 4·5 h. The mixture was then poured into ethanol, stood overnight and decanted. The residue was dissolved in water (400 ml), the pH adjusted to 8 with 5 N NaOH and the solution poured with stirring into ethanol. The precipitate was filtered off and dissolved in 300 ml water. Excess sulfate ions were precipitated with 0·1 N barium acetate solution. Charcoal (30 g) was added and the mixture stirred for 1 h at 50°C and then filtered through Filtrox pad (Filtrox-Werke, St Gallen, Switzerland) and the filtrate precipitated in ethanol. The product was dried *in vacuo* at 60°C (yield, 19 g; M_w, 6900; S, 15·3%; pyridine, 5770 ppm).

Isolation of N-α-D-*xylopyranosyl-pyridinium bromide from an acid hydrolysate of sulfated xylan*

The sulfated xylan (30 g) dissolved in water (250 ml) was passed through a column of Dowex 50 (H+) (3 × 15 cm). The eluate was diluted to *c.* 1:1 with water and heated at 90°C for 17 h. The hydrolysate was neutralized (pH 6) with barium carbonate, filtered and eluted again through the Dowex 50 (H+) column. After washing the resin with water (500 ml) and discarding the washings, 3 M HCl (500 ml) were passed through the column and the acid eluate was collected and evaporated repeatedly with water. The residue was taken up in water (2 ml) to which was then added ethanol (20 ml). After boiling, the solution was filtered and the filtrate evaporated (residue A). Examination of A revealed the presence of both anomers 1 and 2 in the ratio 2:3, respectively. The residue A was converted to the perchlorate form on Dowex I-XI (perchlorate) and fractionated on a cellulose (Avicel) column (35 cm × 3 cm) using *n*-butanol/ethanol/water (40:11:19 v/v) as eluant. Anomer 1 was eluted first and was reconverted to the bromide form which crystallised from ethanol (20 mg) — it was identical (m.p., mixed m.p., ^{1}H-NMR) with an authentic sample.

RESULTS AND DISCUSSION

We have found that several polysaccharides — for example, dextran, hydrolysed waxy maize starch and xylan — when sulfated in the presence

TABLE 1
Pyridinium Contents of Sulfated Polysaccharides[a]

Sample	$\bar{M}_w$	*S%*	*Pyridinium (ppm)*
Dextran	4000	18·8	3410
Waxy maize starch	5200	18·0	1880
Xylan	5000	15·6	2500

[a]The sulfation was performed as described in the Experimental Section.

of pyridine may display UV absorption around 260 nm. The ^{1}H-NMR spectrum of these UV absorbing products reveals three multiplets in the range 8–9 ppm. The coupling constants and shifts are characteristic of the pyridinium moiety. The latter does not seem to be bound ionically since no changes in the UV spectrum are seen after extracting an aqueous solution of the polysaccharide with chloroform at pH 10.

Integration of the pyridinium signals gave contents of up to 10000 ppm in some cases. The pyridinium contents of some polysaccharides prepared under similar conditions are shown in Table 1.

The pyridinium substituents were released only slowly when heated with 2 M HCl. However, with 5 M alkali, high yields of pyridine could be extracted after 2 h.

These properties are consistent with those expected for *N*-glycosyl pyridinium substituents (Hosie *et al.*, 1984) attached to the reducing end-groups of the chains.

We found that the *N*-α-D-xylopyranosyl-pyridinium derivative 1 was stable when heated in 4 M HCl at 100°C for 4 h. This property permitted the isolation of the pyridinium derivative 1 from an acid hydrolysate of a sulfated xylan. After hydrolysis, derivative 1 could be trapped on a Dowex 50 (H+) column and after rinsing with water, was eluted with 3 M HCl. Proton NMR revealed that the acid eluate contained both anomers 1 and 2 in the ratio α:β, 3:2.

The α-anomer of derivative 1 was isolated as its perchlorate salt by chromatography and when reconverted to the bromide form was identical with an authentic sample.

We presume that the pyridine is introduced via displacement of intermediate 1-*O*-sulfate substituents during the sulfation.

REFERENCES

Baba, M., Snoek, R., Pauwels, R. & de Clerq, E. (1988). *Antimicrobial Agents and Chemotherapy*, **32**, 1742.

Grönwall, A., Ingelman, A. & Mosimann, H. F. (1945). *Uppsala Läkareförens. Förh.*, **50**, 397.

Hosie, L., Marshall, P. J. & Sinnott, M. L. (1984). *J. Chem. Soc. Perkin Trans.*, **II**, 1121.

Ohle, H. (1922). *Biochem. Z.*, **131**, 601.

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