Carbohydrate Research, 182 (1988) 166–168
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Structural studies of an extracellular polysaccharide, S-53, elaborated by a *Klebsiella* species

PER-ERIK JANSSON, BENGT LINDBERG, AND GÖRAN WIDMALM
Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 (Sweden)

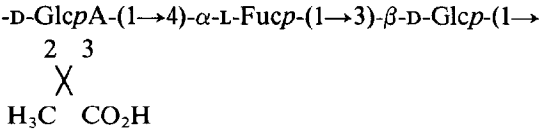
(Received March 15th, 1988; accepted for publication, April 5th, 1988)

During the search for bacteria of potential industrial application, a polysaccharide, S-53, was prepared from a soil bacterium, ATCC No. 31488, identified as a *Klebsiella pneumoniae* species[1]. The polysaccharide had a composition of 18–19% of uronic acid, 4.8% of pyruvate, and 7% of *O*-acetyl, the remainder being glucose and fucose in the ratio 51:49. It was noted that this composition is similar to that reported for the *Klebsiella* type 1 capsular polysaccharide (K1) which, however, does not contain *O*-acetyl groups[2]. We now report structural studies of S-53.

The ^{13}C- and ^{1}H-n.m.r. spectra of S-53 were complex, but the latter contained, *inter alia*, signals for methyl groups of fucosyl residues at δ 1.26 (1 H), of pyruvic acid acetals at δ 1.58 (0.9 H), and of *O*-acetyl groups at δ 2.12 and 2.17 (~3:1, 1 H). The complexity of the spectra may be due to the non-stoichiometric amount of pyruvic acid and the fact that the *O*-acetyl groups occupy different positions.

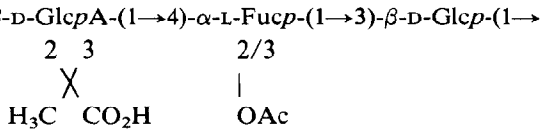
The spectra of *O*-deacetylated S-53 were much simpler and the major peaks were superimposable on those of K1. The pyruvic acid acetals in the *O*-deacetylated S-53 were hydrolysed under mild conditions, typical for such acetals when linked to vicinal *trans*-positions, as in K1[2]. The polysaccharide thus prepared gave 18 well resolved signals in the ^{13}C-n.m.r. spectrum. S-53 and K1 are therefore both composed of the trisaccharide repeating-unit **1**[2], the only difference being that the former contains *O*-acetyl groups. The results of methylation analysis and specific degradations of S-53 confirmed this conclusion. These experiments were similar to those performed[2] in the investigation of K1, and will not be reported in detail here.

0008-6215/88/$03.50



1

Except for the absolute configuration at the acetalic carbon, the only structural feature which remained to be determined was the location of the *O*-acetyl groups. All positions in the β-D-glucopyranosyluronic acid are occupied. A signal at δ 62.1 in the ^{13}C-n.m.r. spectrum of S-53 could be assigned to C-6 of the β-D-glucopyranosyl residue. On acetylation at this position, the signal should move to ~64 p.p.m., but no such signal was present. The signal of C-1 in the α-L-fucopyranosyl residue occurred at δ 100.1 in the spectrum of *O*-deacetylated S-53, but as two signals, at δ 99.9 and 97.5, in the ratio ~2:1, in the spectrum of the original S-53. This is the expected shift on *O*-acetylation at the 2-position in a fucopyranosyl residue[3], and it is concluded that part of this position in S-53 is *O*-acetylated. It was not possible, from the n.m.r. spectra, to decide which of the remaining secondary positions were *O*-acetylated. However, it is most probable that, during the biosynthesis of S-53, a unique position is *O*-acetylated and that the *O*-acetyl group may migrate during manipulations and storing. If this view is correct, the acetyl group should be distributed between O-2 and O-3 of the α-L-fucopyranosyl residue. In agreement with this assumption, all α-L-fucopyranosyl residues were resistant to periodate oxidation, as demonstrated by treatment with periodate and sugar analysis of the recovered polysaccaride. As the pyruvic acid acetals in S-53 are labile, they were probably present originally in a stoichiometrical amount, but have been partially lost. From the results discussed above, it is concluded that S-53 is composed of trisaccaride repeating-units having structure **2**.



2

Several bacterial polysaccarides produced by different strains of the same species have identical carbohydrate backbones, but may differ in the presence or absence of *O*-acetyl groups and also in the percentage and/or location of such groups. This may give them different rheological and immunochemical properties, and such strains have often been classified as different types. When the chemical structures are known and closely similar, it seems to be more convenient to classify them as different sub-types of the same type. We therefore suggest that the *Klebsiella* strain

which produces S-53 is classified as a sub-type of *Klebsiella* type 1.

EXPERIMENTAL

N.m.r. spectroscopy. — N.m.r. spectra of solutions in D_2O were recorded with a JEOL GX-400 instrument. Sodium trimethylsilylpropanoate-d_4 (δ 0.00, ^1H, 50°) and 1,4-dioxane (δ 67.4, ^{13}C, 70°) were used as internal references.

Treatment with periodate. — A solution of S-53 (12 mg) and sodium metaperiodate (40 mg) in 0.1M acetate buffer of pH 4.0 (20 mL) was kept in the dark for 90 h at 4°. Ethylene glycol (0.4 mL) was then added, the solution kept for 3 h, and the polysaccharide recovered by dialysis and freeze-drying. Sugar analysis of this material and the original S-53 gave identical results.

ACKNOWLEDGMENTS

The sample of S-53 was provided by the Kelco Company, San Diego. This work was supported by grants from the National Swedish Board for Technical Development.

REFERENCES

1 U.S. PAT. 4,291,156 (1981), *Chem. Abstr.*, 95 (1981) 185,582u.
2 C. ERBING, L. KENNE, B. LINDBERG, J. LÖNNGREN, AND I. W. SUTHERLAND, *Carbohydr. Res.*, 50 (1976) 115–120.
3 P.-E. JANSSON, L. KENNE, AND E. SCHWEDA, *J. Chem. Soc., Perkin Trans. 1*, (1987) 377–383.