ELSEVIER

Carbohydrate Research 291 (1996) 109–114

CARBOHYDRATE RESEARCH

Selective cleavage of welan gum (S-130) by oxidative decarboxylation with lead tetraacetate

N. Savitri Kumar [a,*], R.M. Sanjaya K. Ratnayake [a], Göran Widmalm [b], Per-Erik Jansson [c]

[a] *Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka*

[b] *Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*

[c] *Clinical Research Centre, Karolinska Institutet, Huddinge Hospital, Novum, S-141 86 Huddinge, Sweden*

Received 4 March 1996; accepted 1 May 1996

Abstract

Oxidative decarboxylation of peracetylated welan gum (S-130) with lead tetraacetate resulted in selective cleavage of the glucuronosidic linkages. Products of the degradation were reduced with sodium borohydride, *O*-deacetylated, and fractionated. Polymeric and oligomeric fractions were separated and analysed by ^{1}H NMR spectroscopy and fast atom bombardment mass spectrometry, and were found to be monomers, dimers, and trimers of the repeating unit. Results show that this method may be used to liberate alditol-terminated multiples of the repeating unit of peracetylated glycuronans by cleavage and degradation of the uronic acid residues. The reaction sequence also confirms the recent finding that welan gum contains repeating units with randomly distributed terminal groups. © 1996 Elsevier Science Ltd.

Keywords: Welan gum; S-130; Lead tetraacetate; Degradation

1. Introduction

Kitagawa and collaborators have used oxidative decarboxylation with lead tetraacetate [$Pb(OAc)_4$] for the selective cleavage of glycosiduronic acid linkages in such glycosides as saponin derivatives [1,2]. A glucuronide saponin was degraded after reaction with lead tetraacetate followed by alkali treatment and the aglycon component in particular was liberated in excellent yield. The glucuronic acid residue was trans-

* Corresponding author.

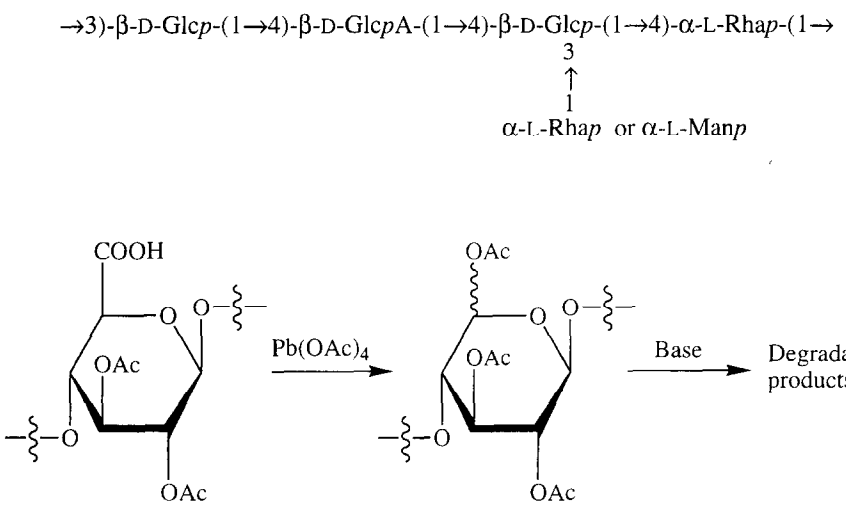
PII S0008-6215(96)00137-1

formed into an epimeric mixture of the corresponding 5-acetoxy-pentopyranoses, which form a pentodialdose component on treatment with alkali. Alkali treatment caused a loss in yield of this component.

Aspinall et al. [3] applied the oxidative degradation with lead tetraacetate to permethylated glycuronans and related model compounds. *O*-Deacetylation and reduction of the 5-acetoxy-pentopyranoses were effected using sodium borohydride without concomitant base-catalysed elimination. The aglycon component as well as the pentitol residues derived from the uronic acid residues were released in good yield. This method was used for the fragmentation under mild conditions of the glucuronomannan chain of methylated Leiocarpan A to yield xylitol-terminated oligosaccharides in which the more acid-labile side-chain residues were still attached [3]. The same reaction sequence was used during the structural analysis of an acidic polysaccharide secreted by a *Xanthobacter sp* [4], to establish the sequence of the glycosyl residues in the tetrasaccharide repeating unit.

The distribution of the terminal L-mannosyl and L-rhamnosyl side-chain groups in welan gum (S-130), an extracellular polysaccharide produced by *Alcaligenes* ATCC 31555, was not determined in an earlier analysis of the polymer [5]. In a more recent study a mixture of oligosaccharides was analysed including a decasaccharide isolated from a partial acid hydrolysis experiment of S-130, and showed that the distribution of the side-chain groups was scrambled [6].

This paper is a report of the lead tetraacetate degradation of peracetylated S-130. The schematic representation of the general degradation procedure is shown in Scheme 1. Selective cleavage of the glucuronosidic linkages was observed and alditol-terminated fragments containing one, two, and three repeating units of S-130 were isolated. The side-chain groups were not cleaved under the reaction conditions used. The structure of welan gum is given by:



Scheme 1. Representation of the lead tetraacetate degradation procedure.

2. Results and discussion

Acetylation of S-130 was carried out with acetic anhydride and pyridine at 80 °C for 24 h to give a 60% yield after workup. Although S-130 was not completely soluble in the acetylating mixture, most of the polysaccharide dissolved during the reaction. Acetylation under the same conditions was repeated if the IR spectrum showed the presence of hydroxyl groups.

The acetylated S-130 was then treated with $Pb(OAc)_4$ in benzene containing a small amount of pyridine and the mixture was refluxed for 4 h at 90 °C. The product obtained after workup was reduced with sodium borohydride (with removal of borate by co-evaporation using 10% acetic acid in methanol followed by methanol), then *O*-deacetylated using aqueous sodium hydroxide, and finally desalted with Amberlite IR-120 (H^+) cation-exchange resin. Products of the reaction were separated by gel filtration on a column of Bio-Gel P-2 which resulted in a polymeric fraction (PS1), two larger oligosaccharides (OS1 and OS2), and two fractions containing tetrasaccharides (OS3 and OS4). The first three fractions were further fractionated on a column of Bio-Gel P-4. The oligosaccharide fractions OS1 and OS2 were of interest because they were identified as trimers and dimers, respectively, of the repeating unit. In the subsequent discussions we will refer to dimers and trimers of the repeating unit, although the oligosaccharides are devoid of one residue.

A hydrolysate of the polymeric fraction PS1 contained rhamnose, mannose, and glucose in the relative proportions 34:9:57. Small amounts of arabinose and xylose were also detected. The native polysaccharide also contained rhamnose, mannose, and glucose, but in the relative proportions 46:11:43. A higher proportion of glucose in the degraded polysaccharide compared to the native polysaccharide can be attributed to release of the β-D-glucopyranosyl residue from the uronic acid residue during the selective cleavage as well as an anticipated higher rate of hydrolysis of the branch-point glucose residue in the modified product.

The fractionated samples were analysed mainly by NMR spectroscopy and FABMS. The ^{1}H NMR spectrum of PS1 showed, inter alia, signals at δ 5.40, 5.28, 5.23, 5.15, 4.75, and 4.54 for anomeric protons. The first four signals correspond to the α-L-mannosyl and α-L-rhamnosyl residues. The two signals at δ 4.75 and 4.54 correspond to β-D-glucuronosyl and β-D-glucosyl residues. The signals at δ ~ 1.3 consisted of doublets due to the methyl protons of rhamnosyl residues. These results indicated that the polymeric fraction isolated after the degradation still carried the side-chain residues.

Sugar analysis of OS1 indicated the presence of rhamnose, mannose, and glucose in the relative proportions 33:14:53. The ^{1}H NMR spectrum of OS1 (Fig. 1) showed signals due to anomeric protons at δ 5.40, 5.28, 5.15, 5.03, 4.75, and 4.55. The signals at δ 5.40, 5.28, 5.15, and 5.03 indicated the presence of α-L-mannosyl and α-L-rhamnosyl residues in the oligomeric fraction. Doublets at δ ~ 1.3 indicated the presence of different groups of methyl protons due to rhamnose residues in the side chain and in the main chain.

The FABMS of OS1 obtained in the positive mode showed three pseudomolecular ions at m/z 2215, 2231, and 2247 which are derived from three repeating units. In agreement with the ratio of terminal rhamnosyl and mannosyl groups in the native

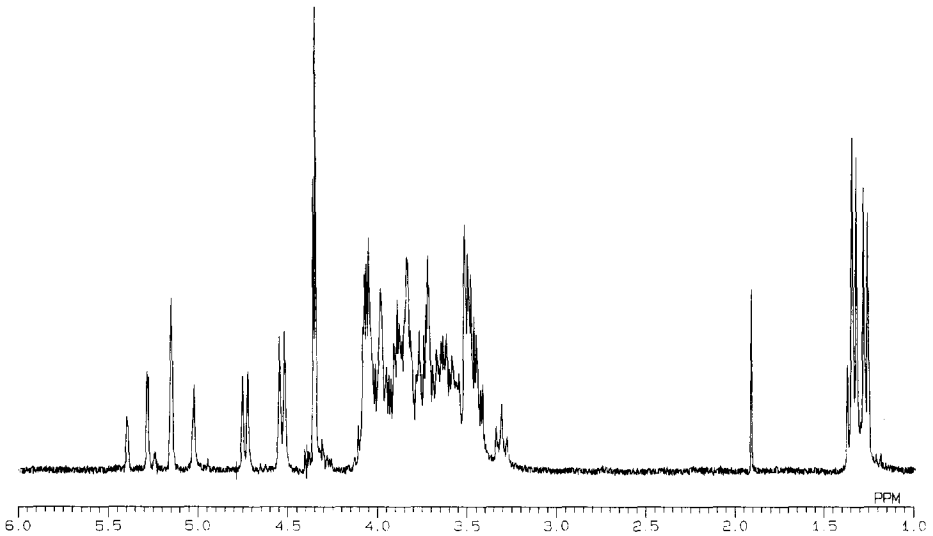


Fig. 1. ^{1}H NMR spectrum at 270 MHz of oligosaccharide mixture OS1.

polymer the intensity of the three peaks decreased with mass number. The most intense peak at m/z 2215 corresponds to a fragment having three side-chain α-L-rhamnosyl groups in three repeating units. The second peak at m/z 2231 corresponds to a fragment with three repeating units carrying two α-L-rhamnosyl groups and one α-L-mannosyl side-chain group, while the peak at m/z 2247 results from a fragment of three repeating units carrying one α-L-rhamnosyl and two α-L-mannosyl side-chain groups, respectively. The structure of the OS1 mixture in shorthand notation is thus:

G-R-G-GA-G-R-G-GA-G-R-Gol
| | |
R/M R/M R/M

where G = glucose, Gol = glucitol, GA = glucuronic acid, R = rhamnose and M = mannose.

The results obtained from the analysis of OS1 confirm the random distribution of the side-chain α-L-rhamnosyl and α-L-mannosyl groups in the repeating unit of the polymer, as observed by differences of 16 amu between the peaks in the FABMS. It would not be possible to obtain fragments containing such combinations of α-L-rhamnosyl and α-L-mannosyl groups in the side chains, if their distribution were regular. A small deviation from the anticipated mass values was observed and may be attributed to reduction/oxidation processes which can occur in FABMS [7].

The sugar composition of OS2 was identical to that of the polymeric fraction PS1 (rhamnose, mannose, and glucose in the relative proportions 34:9:57), and the ^{1}H NMR spectra of OS1 and OS2 were similar. The FABMS of OS2 indicated that it was derived

from two repeating units of the polysaccharide. The smaller fragments, OS3 and OS4, were concluded to be tetrasaccharides by comparing the gel chromatogram with a standard gel chromatogram of a mixture of known oligosaccharides. Sugar analysis, in which the components are derivatised into alditol acetates, of OS3 gave rhamnose and glucose in the ratio 48:52. Hence, this suggested the presence of two rhamnose residues and two glucose residues, and corresponds to a tetrasaccharide alditol, with a rhamnose residue originating from the side chain in the polymer. The ^{1}H NMR spectrum of OS3 showed signals at δ 5.15, 5.04, and 4.73 from anomeric protons, and also showed two doublets at δ 1.36 (3 H) and 1.28 (3 H). The two anomeric signals at δ 5.15 and 5.04 correspond to α-rhamnosidic protons and the two upfield doublets indicated two rhamnose residues. The signal at δ 4.73 was from one of the β-glucose residues since the other glucose residue should be present as an alditol. The shorthand structure of OS3 is thus:

R–G–R–Gol

Sugar analysis, as above, of OS4 gave rhamnose, mannose, and glucose in the relative proportions 26:22:52 and indicated rhamnose, mannose, and two glucose residues in the OS4 sample. The ^{1}H NMR spectrum of the same material showed signals at δ 5.25, 5.04, and 4.73 from anomeric protons, and one doublet at 1.36 (3 H) due to the methyl protons in the rhamnose residue. The anomeric signals at δ 5.25 and 5.04 correspond to α-L-mannosyl and α-L-rhamnosyl residues, respectively. The signal at δ 4.73 was attributed to one β-linked glucosyl residue while the other glucose residue was present as an alditol. Hence this tetrasaccharide alditol is derived from the polysaccharide that has a side-chain mannosyl group. The shorthand structure of OS4 is thus:

M–G–R–Gol

Results from ^{1}H NMR spectroscopy together with sugar analysis indicated that OS3 and OS4 were derived from the two types of monomers of the repeating unit found in S-130. The OS3-type monomer contained one rhamnosyl residue in the main chain and one rhamnosyl group in the side chain. The OS4-type monomer carried one α-L-mannosyl group in the side chain. Hence, both types of monomers as well as dimers and trimers of the repeating unit were obtained from the selective decarboxylation with lead tetraacetate of S-130 polysaccharide.

Therefore, the degradation of S-130 shows that it is possible to isolate the repeating unit of polysaccharides containing uronic acid in the main chain by oxidation of peracetylated derivatives with lead tetraacetate. The polysaccharide was cleaved selectively at the glucuronosyl residue during the reaction and it was possible to isolate the repeating unit and multiples of the repeating unit of the glycuronan using this method.

Many biologically important carbohydrate polymers contain uronic acid residues. The determination of the primary sequence of such polysaccharides is often hampered because the physical properties of the polysaccharide give viscous solutions. In addition the glycosidic bond formed by uronic acid residues is unusually resistant to acid hydrolysis. Methods which result in the cleavage of only selected glycosidic bonds will result in the formation of less complex mixtures, often of oligosaccharides, and make it simpler to deduce the structure of the intact complex carbohydrate. Oxidative decarboxylation of peracetylated glycuronans, such as S-130 with a uronic acid in the backbone,

offers a useful method to obtain underivatised samples of oligomers containing multiples of the repeating unit of these biologically important polymers.

3. Experimental

Acetylation of samples for the degradation.—S-130 (500 mg) was acetylated in Ac_2O (12.5 mL) and pyridine (25 mL) at 80 °C for 24 h. The acetylated polysaccharide was precipitated by dropwise addition of the reaction mixture into cold distilled water (250 mL). The precipitate was dried under vacuum (630 mg) and reacetylated using the same procedure.

Lead tetraacetate degradation.—Acetylated S-130 (30 mg) in benzene (6 mL) containing pyridine (0.2 mL) was treated with $Pb(OAc)_4$ (150 mg) at 90 °C for 4 h. Freshly recrystallised $Pb(OAc)_4$ and dry distilled solvents were used. The cold reaction mixture was diluted with C_6H_6 (6 mL) and filtered. The solution was concentrated to dryness, the residue re-dissolved in CH_2Cl_2 (3 mL), and the CH_2Cl_2 solution extracted with water and evaporated to dryness to yield the reaction product (28 mg).

Reduction and O-deacetylation of products.—Degraded S-130 in 1:1 H_2O–THF (2 mL) was reduced overnight with $NaBH_4$ (30 mg) at room temperature, the mixture was treated with Amberlite IR-120 (H^+) resin, and borate was removed by co-evaporation with methanol. The dry product was *O*-deacetylated with aq 0.1 M NaOH at room temperature overnight.

Analysis of degraded samples.—The products from the degradation of S-130 were fractionated by gel filtration on a column (70 × 2.5 cm) of Bio-Gel P-2 and yielded a polymeric fraction PS1 (5 mg), two oligomeric fractions OS1 (2 mg) and OS2 (1 mg), and two tetrasaccharide fractions OS3 (2 mg) and OS4 (2 mg). The ^1H NMR spectra were recorded on a JEOL GSX-270 spectometer using sodium 4,4-dimethyl-4-sila[2,2,3,3-$^2H_4$]pentanoate as an internal reference ($\delta_H = 0.00$). FABMS were recorded in the positive mode on a JEOL SX-102 instrument and a matrix of 1:1 glycerol–thioglycerol at a resolution of 3000.

Acknowledgements

The authors thank the International Program in Chemical Sciences, Uppsala University, and the Swedish Natural Science Research Council for research grants.

References

[1] I. Kitagawa and M. Yoshikawa, *Heterocycles*, 8 (1977) 783–811.
[2] I. Kitagawa, M. Yoshikawa, and A. Kodata, *Chem. Pharm. Bull.*, 26 (1978) 484–496.
[3] G.O. Aspinall, H.K. Fanous, N.S. Kumar, and V. Puvanesarajah, *Can. J. Chem.*, 59 (1981) 935–940.
[4] M.A. O'Neill, A.G. Darvill, P. Albersheim, and K.J. Chou, *Carbohydr. Res.*, 206 (1990) 289–296.
[5] P.-E. Jansson, B. Lindberg, G. Widmalm, and P.A. Sandford, *Carbohydr. Res.*, 139 (1985) 217–223.
[6] P.-E. Jansson and G. Widmalm, *Carbohydr. Res.*, 256 (1994) 327–330.
[7] K. Vékey, *Int. J. Mass Spectrom. Ion Process.*, 97 (1990) 265–282.