Carbohydrate Research, 94 (1981) 119–122
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Evidence for repeating sub-units in the molecular structure of the acidic arabinogalactan from rapeseed (*Brassica campestris*)

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(Received March 4th, 1981; accepted for publication, March 26th, 1981)

Sequential Smith degradations[1], followed by examination of the products by gel-permeation chromatography, have afforded evidence for the presence of regularly repeating sub-units in the molecular structures of arabinogalactans from the gum exudates of several species of *Acacia*[2–7] and some *Prosopis* species[8], as well as a number of larchwood arabinogalactans[9,10]. The water-soluble polysaccharide isolated by Siddiqui and Wood[11] from rapeseed (*Brassica campestris*) contains residues of L-arabinose, D-galactose, and D-glucuronic acid in the molar ratios of 1:1.05:0.13, and closely resembles these arabinogalactans in its main, structural features: the highly branched polysaccharide molecule is composed of both (1→3)- and (1→6)-linked β-D-galactopyranosyl residues, with terminal L-arabinofuranosyl, D-galactopyranosyl, and D-glucopyranosyluronic acid groups. It therefore seemed of interest to submit this polysaccharide to sequential, Smith degradations in order to ascertain whether similar, repeating sub-units in the D-galactan framework could be recognized.

The sample of polysaccharide used in this investigation was a portion of that isolated and examined by Siddiqui and Wood[11]. Gel-permeation chromatography confirmed the homogeneity suggested by electrophoretic and sedimentation analysis in the earlier study[11], and indicated a value of ~94,000 for the weight-average, molecular weight, $\overline{M}_w$.

The results of a sequence of three, successive, Smith degradations performed on this arabinogalactan are shown in Table I. The value found here for the periodate consumption of the polysaccharide is close to that reported by Siddiqui and Wood[11]. These workers did not isolate the Smith-degraded polysaccharide; the product of reduction by sodium borohydride of the periodate-oxidized arabinogalactan was hydrolyzed with 0.5M sulfuric acid for 5 h at 100°, and the hydrolyzate analyzed by g.l.c., which indicated a galactose:arabinose ratio of ~20:1 and a high proportion

0008-6215/81/0000-0000/$ 02.50,

TABLE I

RESULTS OF SEQUENCE OF THREE SMITH DEGRADATIONS PERFORMED ON THE ACIDIC ARABINOGALACTAN FROM *Brassica campestris*

	Smith degradation		
	1	*2*	*3*
Sample mass (mg)	60	17.8	5.5
Yield of degraded polysaccharide (mg)	18.5	6.8	N.d.[a]
Periodate consumed[b] (mmol.g^{-1})	6.0	2.1	N.d.
Properties of degraded polysaccharide			
$[\alpha]_D$ (degrees)[c]	+14	+20	N.d.
Molecular weight[d]	6300[e]	5500[e]	5000[e]

[a]N.d. = not determined. [b]By arsenite method, modified as described in text. [c]Concentration, 0.45%. [d]From gel-permeation chromatography on Bio-Gel P-10, calibrated with dextrans. [e]Single, sharp peak.

(~43%) of glycerol. In the present work, analysis of a hydrolyzate of the polysaccharide product (SD1) of the first Smith degradation was omitted, in order to maximize the quantity available for further degradations of this type, but the proportion of the methanol-soluble fraction (44% of the total products of the Smith degradation), which was found (by paper chromatography) to consist mainly of glycerol, was in accordance with the earlier analysis[11]. No sugars were detected in the latter fraction. The results of hydrolysis of a sample of the polysaccharide (SD2) produced by the second, sequential, Smith degradation indicated that this was composed almost entirely of galactosyl residues. A small trace of arabinose was detected, both in this hydrolyzate and in the methanol-soluble fraction (mostly glycerol) of the Smith-degradation products.

The formation of apparently monodisperse polysaccharides in all three Smith degradations strongly suggests that the *B. campestris* arabinogalactan resembles those found in larch heartwood, and gums of *Acacia* and *Prosopis* species, in having a molecular structure composed of regularly repeating sub-units. Methylation analysis[11] indicated that ~50% of the sugar residues constituting the polysaccharide molecule are removed after one Smith degradation and, therefore, the molecular weight (~6000) of the SD1 product suggests a sub-unit having a molecular weight of ~12,000 in the intact polysaccharide. Sub-units of similar size have been postulated for several of the plant-gum arabinogalactans[5-8]. The small decreases in molecular weight accompanying the second and third Smith degradations are consistent with the removal of a few terminal residues only, from which it may be inferred that SD1 is composed largely of the periodate-resistant, (1→3)-linked, D-galactopyranosyl residues constituting the repeating blocks in the D-galactan framework of the polysaccharide.

EXPERIMENTAL

General methods. — The solvent systems used in paper chromatography were (v/v): (*1*) 8:2:1 ethyl acetate–pyridine–water; and (*2*) 4:1:5 (upper layer) 1-butanol–ethanol–water. Sugars and polyols were distinguished on papers by use of both the *p*-anisidine hydrochloride and ammoniacal silver nitrate spray-reagents.

Gel-permeation chromatography[12] of the arabinogalactan and the polysaccharides produced on Smith degradation was performed on columns of Bio-Gel P-300 and Bio-Gel P-10, respectively.

Isolation and purification of polysaccharide. — The isolation and fractionation of the acidic arabinogalactan from the mixture obtained from rapeseed has been fully described[11,13,14].

Smith degradations. — In all three of the sequential, Smith degradations, the polysaccharides were oxidized with 0.12M sodium metaperiodate for 72 h. At the first and second degradation stages, the consumption of periodate was determined by the standard arsenite method[15], modified[8] by use of graduated syringes to permit the titration of very small aliquots (30–50 μL), but, in the third degradation, scarcity of material precluded this determination. The method used to terminate oxidation varied with the molecular size of the polysaccharide oxidized and the quantity available: in the first Smith degradation, the excess of periodate was allowed to react with ethylene glycol and the solution was then dialyzed, as before[11], but, in the second, periodate was removed by precipitation with barium acetate[3]. In both of these degradations, reduction of the oxidized polysaccharide with sodium borohydride was followed by removal of borate, and de-ionization with ion-exchange resins in the usual way[2,11], before the product was submitted to mild hydrolysis with M trifluoroacetic acid during 48 h at 20°. In the third, however, the paucity of material necessitated the omission of several steps, in order to minimize losses. After termination of oxidation by addition of ethylene glycol (monitored by spot tests with silver nitrate solution, with which iodate and periodate give white and dark-brown precipitates, respectively), sodium borohydride was added directly to the resulting solution. Reduction was continued for 72 h, and the solution was then acidified with trifluoroacetic acid and immediately freeze-dried. Borate was removed from the residue by evaporation with methanol, but de-ionization with resins was omitted, and the entire residue was used in the mild-hydrolysis step.

The duration of the treatment of the reduced, oxidized polysaccharide with M trifluoroacetic acid at room temperature was decided, in the first Smith degradation, by monitoring the hydrolysis by gel-permeation chromatography of small samples removed at intervals, as before[2–8]. By this criterion, Smith degradation was complete after mild hydrolysis for 24 h: no change in the molecular weight of the degraded polysaccharide was observed on continuation of the treatment with acid for a further 24 h. The period of 48 h allowed for this process during the subsequent, Smith degradations was, therefore, considered more than sufficient to ensure complete cleavage of vulnerable linkages.

The products of the first and second Smith-degradations were recovered by fractionation with methanol in the usual way[2,3]. The methanol-soluble fractions were examined by paper chromatography in solvents *1* and *2*. Hydrolysis (2M trifluoroacetic acid, 18 h at 100°) of a small sample (~1 mg) of the degraded polysaccharide SD2 was followed by paper chromatography in solvent *1*. In the third Smith degradation, no products were isolated: after the mild-hydrolysis step, all of the reaction mixture (1 mL) was made neutral with sodium hydrogencarbonate and then applied to the Bio-Gel P-10 column, on which the degraded polysaccharide was well separated from the inorganic contaminants.

ACKNOWLEDGMENTS

The financial support of the South African Council for Scientific and Industrial Research and the University of Cape Town is gratefully acknowledged.

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