505. *The Catalytic Oxidation of European Larch ε-Galactan.**

By G. O. Aspinall and A. Nicolson.

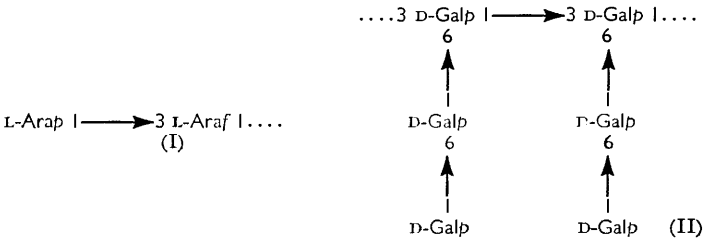
Catalytic oxidation of larch ε-galactan results in selective oxidation of primary alcoholic groups with the formation of carboxylic acids. Graded hydrolysis of the oxidised polysaccharide gives two aldobiouronic acids, (6-D-galactose β-D-galactopyranosid)uronic and (6-D-galactose L-arabinofuranosid)uronic acid. The structural significance of these and other results is discussed.

Previous investigations [1] have shown that the arabinogalactan (ε-galactan) from European larch wood (*Larix decidua*) is a highly branched polysaccharide containing L-arabinose and D-galactose residues in the approximate proportion of 1 : 6. The majority of arabinose residues are accommodated as 3-*O*-β-L-arabinopyranosyl-L-arabinofuranose units (I). Since a polysaccharide cannot be built up from such units alone, it was concluded that the arabinose residues were constituent parts of an arabinogalactan, but direct proof was not available. The highly branched framework of the polysaccharide is composed of D-galactopyranose residues with the main chains containing 1,3-linkages and the outer chains containing 1,6-linkages. The partial structure (II) represents one way in which a large proportion of the galactose residues in the polysaccharide may be accommodated. The partial structures (I and II) do not take into account the mode of attachment of arabinose residues in the side-chains (I) to galactose and, therefore, do not include the galactose residues (approximately 1 in 12) which are involved at these points. The previous methylation studies, however, indicated a proportion of branching points in the polysaccharide in excess of those required by that part of the molecular structure represented by (II). Evidence for the mode of attachment of L-arabinose to D-galactose

* A summary of this paper was presented at the Atlantic City meeting of the American Chemical Society, September, 1959.

[1] Aspinall, Hirst, and Ramstad, *J.*, 1958, 593 and references then cited.

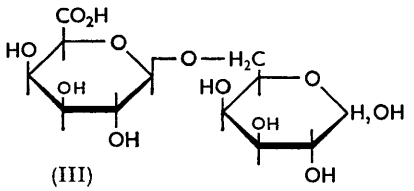
residues in the polysaccharide has hitherto been lacking since L-arabinofuranose residues are readily cleaved by acid and it has been impossible to isolate, as products of partial hydrolysis, oligosaccharides containing both arabinose and galactose residues. This paper describes a new approach to the problem.



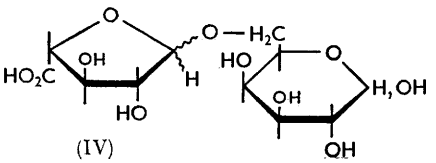
(I) (II)

We have recently shown [2] that the primary alcoholic groups of polysaccharides may be oxidised to carboxylic acids by Mehltretter's procedure which had been developed for the preparation of glycosiduronic acids.[3] The oxidation is effected by passing oxygen through an aqueous solution of the polysaccharide, containing sufficient sodium hydrogen carbonate to neutralise acid formed, in the presence of a platinum catalyst. Both glycofuranosiduronic and glycopyranosiduronic acid linkages in the oxidised polysaccharides,[2] thus formed, resist acid-hydrolysis, and aldobiouronic acids may be isolated as products of graded hydrolysis. The larch arabinogalactan contains terminal D-galactopyranose and non-terminal L-arabinofuranose residues susceptible to oxidation, and treatment of the polysaccharide in this way resulted in the oxidation of a proportion of the available alcoholic groups with the formation of an acidic polysaccharide with an uronic anhydride content of 7·5%. Hydrolysis of the oxidised polysaccharide, followed by chromatographic separations of the products on charcoal–Celite and on filter sheets led to the isolation of two acidic oligosaccharides.

The acidic oligosaccharide formed in largest amount was shown to be (6-D-galactose β-D-galactopyranosid)uronic acid (III) by the following experiments. The reducing power, measured by hypoiodite oxidation, showed the compound to be a disaccharide. Reduction of the derived methyl ester methyl glycosides with potassium borohydride, followed by hydrolysis, gave galactose only. Oxidation of the disaccharide with periodate gave only traces of formaldehyde, whereas oxidation of the acidic disaccharide alcohol (from reduction by borohydride) gave 0·85 mol. of formaldehyde, a result consistent only with the presence of a 1,5- or a 1,6-linkage. Conclusive evidence for a 1,6-linkage was obtained by reduction of the methyl ester methyl glycosides to the corresponding mixture of methyl glycosides of a neutral galactobiose, which on oxidation consumed 3·8 mol. of periodate with the liberation of 1·8 mol. of titratable (presumably formic) acid.



(III)



(IV)

The following experiments indicated that the second acidic oligosaccharide was (6-D-galactose L-arabinofuranosid)uronic acid (IV). The reducing power, by hypoiodite oxidation, showed the compound to be a disaccharide. Reduction of the derived methyl ester methyl glycosides with potassium borohydride, followed by hydrolysis, gave arabinose and galactose. Since treatment of the disaccharide itself with potassium borohydride

[2] Aspinall, Cairncross, and Nicolson, *Proc. Chem. Soc.*, 1959, 270.
[3] Mehltretter, *Adv. Carbohydrate Chem.*, 1953, **8**, 231.

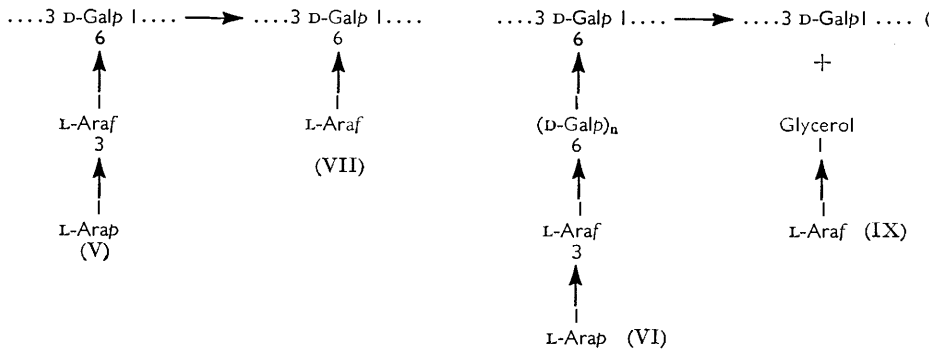
reduced the galactose residue, it follows that the compound was a (galactose arabinosid)-uronic acid. Oxidation of the disaccharide with periodate gave only traces of formaldehyde, whereas oxidation of the disaccharide alcohol (from reduction by borohydride) gave 0·87 mol. of formaldehyde. It follows that the disaccharide contains a 1,5- or a 1,6-linkage. Since previous experiments [1] provided no evidence for the presence in the polysaccharide of 1,5-linked D-galactose residues, necessarily present in the furanose form, it is reasonably certain that the aldobiouronic acid must contain a 1,6-linkage.

The isolation of the aldobiouronic acid, (6-D-galactose β-D-galactopyranosid)uronic acid (III), on hydrolysis of the oxidised ε-galactan provides additional proof to that already put forward [1] that the D-galactopyranose end groups in the polysaccharide are joined to adjacent D-galactose residues by 1,6-linkages. The formation of the second aldobiouronic acid, (6-D-galactose L-arabinofuranosid)uronic acid (IV), shows that the 3-*O*-β-L-arabinopyranosyl-L-arabinofuranose side-chains (I) are attached to position 6 of D-galactose residues.

On the basis of this evidence, two alternative partial structures (V and VI) may be advanced to indicate the attachment of arabinose to galactose residues in the polysaccharide; these structures include only those galactose residues directly involved in linking arabinose residues to the main chains of the polysaccharide and are additional to the major part of the galactan framework as represented by (II) with which they may be combined so that the polysaccharide as a whole contains arabinose and galactose residues in the approximate proportions of 1 : 6. In structure (V), arabinose residues are joined directly, as side-chains, to position 6 of 1,3-linked galactose residues in the main chains of the polysaccharide, whereas in structure (VI), arabinose residues terminate side-chains containing one or more 1,6-linked galactose residues. Evidence in favour of the partial structure (V) as the most important mode of attachment of arabinose to galactose residues has been obtained by the application to the polysaccharide of the elegant degradative method recently described by F. Smith and his collaborators.[4] Reduction of the periodate-oxidised ε-galactan with potassium borohydride, followed by controlled hydrolysis with cold N-sulphuric acid, gave a degraded polysaccharide together with products of low molecular weight. Hydrolysis of the degraded polysaccharide gave galactose and appreciable amounts of arabinose. The isolation of such a degraded polysaccharide in which arabinose residues are still linked to galactose would point to the partial structure (VII) as a fragment of the molecule. A polysaccharide containing arabinose residues linked to galactose as in partial structure (VI) would be degraded to a galactan devoid of arabinose residues as indicated by (VIII) which shows only the galactose residue to which the arabinose-containing side-chain was formerly attached. In this case the non-terminal arabinose residues in the polysaccharide, which would not be attacked by periodate, would give rise to L-arabinofuranosylglycerol (IX) as a degradation product. Chromatographic examination of the products of low molecular weight from the degradation showed that very small amounts of a non-reducing substance tentatively identified as an arabinosyl-glycerol. Although no quantitative estimation of arabinose-containing degradation products was made, the results indicate that the greater proportion of the arabinose residues in the polysaccharide, which were unattacked by periodate, was still linked to the galactan framework in the degraded polysaccharide. It is probable, therefore, that the majority of the 3-*O*-β-L-arabinopyranosyl-L-arabinofuranose units are attached directly as side-chains to position 6 of galactose residues in the 1,3-linked main chain as in (V), although it is possible that a small proportion of these groups may be attached to galactose in other ways (*e.g.*, VI). Our earlier observation [1] that degradation of the periodate-oxidised ε-galactan with phenylhydrazine by Barry's method [5] led to a degraded polysaccharide containing both arabinose and galactose residues is in agreement with the present results.

[4] Goldstein, Hay, Lewis, and Smith, Amer. Chem. Soc. Meeting, Boston, April 1959, Abs. Papers, 3 D.

[5] Barry and Mitchell, *J.*, 1954, 4020.



After this work had been completed, Professor J. K. N. Jones, F.R.S. (personal communication), informed us that he had reached similar conclusions concerning the mode of attachment of arabinose to galactose residues in ε-galactan. His conclusions were based on an examination of the degraded galactan (VII) formed by Smith's degradative procedure and of a degraded 1,3-linked galactan, devoid of arabinose residues, formed after a second degradation. We are grateful to Professor Jones for supplying us with this information in advance of its publication.

Experimental

Paper chromatography was carried out on Whatman Nos. 1 and 3MM papers with the following solvent systems (v/v): (A) ethyl acetate–acetic acid–water (3 : 1 : 3, upper layer); (B) ethyl acetate–pyridine–water (10 : 4 : 3); (C) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4). Filter-sheet separations on 3MM paper were effected on paper which had been previously extracted with 8-hydroxyquinoline in acetone. Reducing sugars were detected by spraying chromatograms with saturated aqueous aniline oxalate, and non-reducing polyhydroxy-compounds with ammoniacal silver nitrate.

Catalytic Oxidation of ε-Galactan and Hydrolysis of the Oxidised Polysaccharide.—A number of preliminary experiments indicated that oxidation of polysaccharides was slow when carried out under the general conditions described below, and that even after long periods oxidation of the available primary alcoholic groups was incomplete. Under similar conditions oxidation of simple sugar derivatives, *e.g.*, methyl α-D-mannopyranoside, was complete after 12—24 hr.[1]

Solutions of ε-galactan (5 g.) in water (150 ml.) and of sodium hydrogen carbonate (0·5 g.) in water (100 ml.) were each shaken with platinum catalyst (Adams platinic oxide after reduction with hydrogen; 10 mg.) to remove possible poisons, the platinum was removed at the centrifuge and the solutions were combined. Platinum catalyst (1 g.) was added to the solution and oxygen was bubbled through the stirred mixture held at 70° for 14 days, water being added when necessary to compensate for evaporation losses. Platinum was removed from the cooled solution by centrifugation, the solution was concentrated, and the oxidised polysaccharide was precipitated by the addition of ethanol (3 vol.). The polysaccharide was dissolved in water, the solution was passed through a column of Amberlite resin IR-120(H^+) to remove sodium ions, and the acidic polysaccharide (3·2 g.) was precipitated by the addition of ethanol [Found: uronic anhydride (Kaye and Kent's method [6]), 7·5%]. Hydrolysis of a sample of the acidic polysaccharide gave two acidic oligosaccharides ($R_{galactose}$ 0·5 and 0·25 in solvent A) which were separated from neutral sugars (arabinose and galactose) by absorption on Amberlite resin IR-45(OH^-).

The acidic polysaccharide (3 g.) was hydrolysed by N-sulphuric acid (50 ml.) at 100° for 4 hr. Sulphuric acid was removed from the hydrolysate by shaking it with methyldi-n-octylamine (5% v/v in chloroform; 4 extractions), and the aqueous solution was poured on to charcoal–Celite (1 : 1; 15 g.). Elution with water gave arabinose and galactose, and elution with water containing 20% of ethanol gave a complex mixture of sugars (330 mg.). Since chromatography showed several oligosaccharides, both neutral and acidic, the mixture was rehydrolysed with N-sulphuric acid (30 ml.) at 100° for 2 hr. The hydrolysate was treated as before to remove

[6] Kaye and Kent, *J.*, 1953, 79.

sulphuric acid, and the resulting syrup was fractionated on filter sheets with solvent A, to give galactose and two acidic oligosaccharides.

Examination of Acidic Oligosaccharides.—Fraction 1. The syrup (95 mg.) had $R_{galactose}$ 0·25 in solvent A and reducing equivalent (by hypoiodite oxidation [7]), 341 [calc. for (hexose hexosid)uronic acid, 358]. Conversion into the methyl ester methyl glycosides, reduction with potassium borohydride, and hydrolysis gave galactose only (chromatography). Oxidation of the sugar with sodium metaperiodate in the presence of sodium hydrogen carbonate gave 0·04 mol. of formaldehyde (estimation by the method of O'Dea and Gibbons [8]). Reduction of the sugar with potassium borohydride, followed by oxidation with periodate, gave 0·87 mol. of formaldehyde. The sugar (55 mg.) was converted into the methyl ester methyl glycoside by boiling with methanolic hydrogen chloride (50 ml.) for 6 hr. The ester glycosides were treated with potassium borohydride (40 mg.) in water (10 ml.) for 24 hr., and the mixture of methyl glycosides of the neutral disaccharide (27 mg.) was isolated after chromatography on filter sheets in solvent B. The methyl glycosides (5 mg.) were oxidised with sodium metaperiodate (0·015M; 10 ml.) at 35°. The uptake of periodate, measured spectrophotometrically [9] and constant after 10 hr., corresponded to 3·8 moles per mole of methyl galactobioside. The methyl glycosides (20 mg.) were dissolved in 0·56M-potassium chloride (50 ml.), 0·2M-sodium metaperiodate solution (20 ml.) was added, and the mixture was left in the dark. Aliquot parts (10 ml.) were withdrawn at intervals, excess of periodate was destroyed by ethylene glycol (1 ml.), and the liberated acid was titrated with 0·015N-sodium hydroxide. The titratable acid (presumably formic acid) released was constant after 10 hr., and corresponded to 1·8 moles per mole of methyl galactobioside.

Fraction 2. The syrup (45 mg.) had $R_{galactose}$ 0·5 in solvent A and reducing equivalent (by hypoiodite oxidation) 314 [calc. for (hexose pentosid)uronic acid, 328]. Conversion into the methyl ester methyl glycosides, reduction with potassium borohydride, and hydrolysis gave arabinose and galactose (chromatography). Similar treatment of the disaccharide alcohol (from reduction by potassium borohydride) gave only arabinose. Oxidation of the sugar with periodate gave 0·06 mol. of formaldehyde. Reduction of the sugar with potassium borohydride followed by oxidation with periodate gave 0·87 mol. of formaldehyde.

Preparation and Mild Hydrolysis of Reduced Periodate-oxidised ε-Galactan.—ε-Galactan (7 mg.) was oxidised with 0·2M-sodium metaperiodate (480 ml.) for 144 hr.[1] The solution was treated with lead acetate to remove iodate and periodate, and then with dilute sulphuric acid to precipitate the excess of lead. The clarified solution was neutralised with methyldi-n-octylamine (5% v/v in chloroform). Potassium borohydride (4 g.) was added to the solution of the periodate-oxidised polysaccharide and, after 3 days, excess of borohydride was destroyed by treatment with Amberlite resin IR-120(H^+), the solution was concentrated, and the reduced periodate-oxidised polysaccharide (3·5 g.) was precipitated by the addition of acetone (3 vol.).

Reduced periodate-oxidised polysaccharide (3·5 g.) was dissolved in N-sulphuric acid at room temperature in 30 min.; the solution was then neutralised with barium carbonate and afforded degraded polysaccharide (0·92 g., precipitated with 3 vol. of acetone) and non-reducing syrup (1·0 g.). Hydrolysis of the degraded polysaccharide gave arabinose and galactose in the approximate proportion of 1 : 5 (as judged from relative intensities of spots on chromatograms). The syrup (1·0 g.) was absorbed on a column of charcoal–Celite; elution with water afforded fraction (i) (0·9 g.), and elution with water containing 15% of ethanol afforded fraction (ii) (*ca.* 20 mg.). Chromatography of fraction (i) in solvent C showed glycerol as the major component with traces (only detected on heavy spotting of chromatograms) of a non-reducing substance ($R_{arabinose}$ 1·4) and of arabinose. Chromatography of fraction (ii) showed a main component with $R_{arabinose}$ 1·4 in solvent C which gave arabinose and glycerol on hydrolysis.

In a control experiment, treatment of ε-galactan with N-sulphuric acid at room temperature for 30 min. afforded no detectable reducing sugars.

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[7] Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289.
[8] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.
[9] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.