ACTA CHEMICA SCANDINAVICA 10 (1956) 1515—1519

Studies on Arabogalactans

I. Products from the Mild Hydrolysis of the Arabogalactan from *Larix occidentalis*

HANS BOUVENG and BENGT LINDBERG

Organisk-kemiska Institutionen och Träkemiska Institutionen, Kungl. Tekniska Högskolan, Stockholm, Sweden

The arabogalactan from *Larix occidentalis* yielded a mixture of arabinose, galactose, two disaccharides and a small trisaccharide fraction when hydrolysed under conditions mild enough to cleave furanosidic linkages almost exclusively. One of the disaccharides was shown to be 3-β-L-arabopyranosyl-L-arabinose which had previously been obtained from a similar polysaccharide by Jones [1]. The other disaccharide was demonstrated to be 6-β-D-galactopyranosyl-D-galactose. The trisaccharide mixture was not further fractionated but on partial hydrolysis the arabopyranosyl-arabinose referred to above was obtained, together with arabinose and galactose.

The water-soluble polysaccharides from species of the *Larix* genus have been subject of several investigations. The literature up to 1952 has been summarised by Whistler and Smart [2]. The polysaccharide is branched and built up from 1,3′- and 1,6′-β-linked D-galactopyranosidic units and terminal L-arabofuranosidic units. Jones [1] isolated a disaccharide, 3-β-L-arabopyranosyl-L-arabinose, after mild hydrolysis of the arabogalactan from *L. decidua*. Considering the mild conditions during the hydrolysis, Jones assumed, that this disaccharide, as well as the free arabinose formed at the same time, is linked through a furanosidic linkage to the rest of the polysaccharide. He indicated the possibility that the free galactose, which is formed in small amounts under these mild conditions, should possess a furanosidic linkage also.

Sedimentation analyses showed that the polysaccharide from *L. occidentalis* consisted of two fractions with molecular weights of 16 000 and 100 000, respectively. Lystad Borgin [3] however, demonstrated that the heartwood contained only the fraction of the highest molecular weight, while both fractions were present in the sapwood.

Polysaccharides of this type are probably present in all Conifers, as indicated by their high percentage of galactose, and an arabogalactan has recently been isolated from *Pinus Jeffreyi* by Wadman, Anderson and Hassid [4].

The present paper is the first in a series with the aim of studying the arabogalactans in coniferous woods. The water-soluble polysaccharide from *L. occidentalis* was subjected to mild hydrolyses, first with 0.01 N hydrochloric acid at 100°C for two hours and then, after separation of the sugars and oligosaccharides, with 0.02 N hydrochloric acid at 100°C for two hours. Even after the last treatment the polysaccharide contained small amounts of arabinose. Chromatograms of the hydrolysates showed the presence of arabinose and galactose in dominating quantities, two disaccharides and traces of substances in the trisaccharide region. As the products from the two hydrolyses gave essentially the same chromatographic picture, they were combined and separated on a carbon column. L-Arabinose (5.9 %), D-galactose (2.2 %), two disaccharides, I (2.0 %) and II (0.8 %), and a mixture of trisaccharides (0.8 %) were isolated. The yield of higher oligosaccharides, eluted from the column with 50 % ethanol, was 2.5 %. The nature of these oligosaccharides is now under investigation.

Considering the high proportions of low-molecular weight saccharides formed under conditions when the velocity constants for hydrolysis of glycosidic linkages (expressed in minutes and Briggs logarithms) are about 10^{-4} for pyranosides and 10^{-1} — 10^{-2} for furanosides [5], it is strongly indicated, that these saccharides are linked to the rest of the molecule through furanosidic linkages, and that only a minute amount of pyranosidic linkages are cleaved during these mild conditions.

On hydrolysis, disaccharide I yielded arabinose only and disaccharide II galactose only. After methylation and hydrolysis, disaccharide I yielded a mixture of two substances, which were separated on a carbon column. One of these was identified as 2,3,4-tri-*O*-methyl-L-arabinose by transferring it into the amide of the corresponding arabonic acid, identical with an authentic specimen. The other substance was identified as 2,4-di-*O*-methyl-L-arabinose. This substance has not yet been synthesised, but its identity has been unambiguously proved [1,6], and it was now characterised as the N-phenylamine derivative and as the amide of the corresponding arabonic acid. The disaccharide therefore is a 3-L-arabopyranosyl-L-arabinose, and from its specific rotation +200°, the β-configuration of the pyranosidic linkage is highly probable. Thus it is identical with the disaccharide isolated by Jones [1] from the arabogalactan obtained from *L. decidua*.

On methylation and hydrolysis of the other disaccharide (II), a mixture of 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-galactose was obtained. They were separated by chromatography on thick filter paper and characterised as their N-phenylamine derivatives, identical with authentic specimens. The disaccharide therefore is a 6-D-galactopyranosyl-D-galactose The specific rotation, +30°, indicates a β-pyranosidic structure.

The trisaccharide fraction on total hydrolysis yielded a mixture of arabinose and galactose. On partial hydrolysis with 0.01 N hydrochloric acid for one hour at 100° a substance, chromatographically indistinguishable from disaccharide I above, was also obtained. This indicates that, in at least one of the trisaccharide components, the 3-L-arabopyranosyl-L-arabinose is a structural unit with a furanosidic linkage to either arabinose or galactose.

The rather simple picture of the arabogalactan given by previous investigators was complicated by Jones [1], who demonstrated the presence of

arabopyranosidic units in the molecule. The results of the present investigation introduce further complications. It is not yet settled, whether the polysaccharide is homogeneous or consists of a mixture of a galactan and an arabogalactan; the results of previous sedimentation studies [3] indicate, however, that the material here investigated should be homogeneous. In the highly branched framework of 1,3′- and 1,6′-linked β-galactopyranosidic units, the arrangement of which is not known, arabinose, galactose and di- and higher saccharides of these sugars are linked to the rest of the molecule through furanosidic linkages. In these saccharides some of the linkages between the monomers are pyranosidic, 1,3′-bonds for arabinose to arabinose and 1,6′-bonds for galactose to galactose being demonstrated. Other bonds, however, must be furanosidic, as indicated by the results from the partial hydrolysis of the trisaccharide fraction. The amount of easily hydrolysed parts of the molecule may be estimated at about 25 % of the polysaccharide.

EXPERIMENTAL

All melting points corrected. All evaporations performed under reduced pressure.

Chromatography. Papers: Whatman No. 1 and 3MM. Solvent: Butanol, ethanol, water, 10:3:5. Developers: Silver nitrate/sodium ethoxide and anisidine hydrochloride.

Preparation of arabogalactan. Ground heartwood of *Larix occidentalis* (580 g) was continuously extracted with ether (30 h) and subsequently with methanol (90 h). The wood (540 g) was then extracted with cold water (4 + 2 + 2 l) for 12 – 18 h with continuous stirring. The extracts were separated from the wood by filtering through a cotton cloth and then through paper, using a filter aid (Celite). The extracts were concentrated until they became viscous and then precipitated in anhydrous ethanol. The sticky precipitate was washed with anhydrous ethanol and ether and dried in a desiccator, yielding a light-coloured powder (46.5 g, 8.6 %). $[\alpha]_D^{20}$ + 7.5° (*c*, 2.0 in water).

Partial hydrolysis of arabogalactan. The polysaccharide (25 g) was treated with 0.010 N hydrochloric acid (650 ml) at 100°C for 2 h. After being cooled, the solution was neutralised by filtering through a column of IR 4B and then concentrated to a viscous liquid, which was poured into ethanol to a concentration of 90 % ethanol. The precipitate formed was filtered off and the filtrate treated with active carbon in 50 % ethanol to free it from dextrins and then filtered and concentrated to a colourless syrup (1.42 g).

The precipitate mentioned above was treated with 0.020 N hydrochloric acid (600 ml) at 100°C for 2 h. After neutralisation (IR 4B) and concentration the product was precipitated in ethanol at 90 % concentration and filtered. The precipitate was dissolved in water and the procedure repeated. The filtrates (2.15 and 0.89 g, resp. when concentrated) were combined and yielded, after being treated with carbon in the same manner as described above, a colourless syrup (3.08 g) upon concentration. The amount of high molecular weight material, recovered after the last hydrolysis, was 20.0 g.

Separation of the products from the partial hydrolyses of arabogalactan. Column: 4.5 × × 33 cm. Eluent: 8 l 0 – 20 % ethanol. Fractions: *ca.* 150 – 100 ml. Added amount: 4.50 g.

Fract. 10 – 15. Arabinose (1.47 g). M.p. 157 – 158°. $[\alpha]_D^{20}$ +104° (*c*, 2.0 in water)

» 16 – 21. Galactose (0.54 g). M.p. 164 – 165° $[\alpha]_D^{20}$ + 82° (*c*, 2.0 in water)

» 32 – 37. 0.51 g of an amorphous disaccharide (I), giving a carmine colour with anisidine hydrochloride and on total hydrolysis arabinose only. $[\alpha]_D^{20}$ + 212° (*c*, 2.0 in water).

» 38 – 46. 0.20 g of an amorphous disaccharide (II) (traces of (I) in fract. 38), giving a brown-yellow colour with anisidine hydrochloride and on total hydrolysis galactose only. $[\alpha]_D^{20}$ + 30° (*c*, 2.0 in water).

» 54 – 70. 0.19 g of at least two trisaccharides.

Elution with 50 % ethanol yielded on concentration 0.63 g of higher saccharides. The recovery of 3.48 g of an in all added 4.50 g shows that the added material was not dry.

Methylation of (I) and hydrolysis of the methylated products. 0.51 g of (I) was dissolved in water (5 ml) and methyl sulphate (1 ml) added, the solution being vigorously stirred and the temperature kept at 0°C. During 7 h 30 % sodium hydroxide (2 ml) was added dropwise, and stirring continued for 4 h more, when all methyl sulphate had dissolved. The reaction mixture was then deionised (IR 120 followed by IR 4B).

The product was concentrated and water removed by repeated distillation, first with anhydrous chloroform and then with dimethyl formamide. Complete methylation was performed by adding methyl iodide (2.5 ml) and silver oxide (2.5 g) to a rapidly stirred solution of the premethylated disaccharide in dimethyl formamide (10 ml), the apparatus and reagents being previously carefully dried and the reaction mixture kept below 20°C by external cooling. The silver oxide was added during one hour and the stirring continued for another 11 h. The reaction product was worked up according to Kuhn *et al.*[7]. Yield: 0.56 g (87 %).

The methylated disaccharide was hydrolysed in 0.5 N hydrochloric acid at 100° for 12 h. After neutralisation (IR 4B) the solution was concentrated, yielding a light brown syrup (0.50 g).

Separation on a carbon column of the products from the methylated and hydrolysed disaccharide (I). Column: 3 × 20 cm. Eluent: 4 l. 10 — 40 % ethanol. Fractions: 26 ml. Added amount: 0.50 g.

Fract. 8 — 14. 0.18 g of a substance with a rather high R_F-value (Ia).
» 17 — 32. 0.21 g of a substance with a somewhat higher R_F-value (Ib).

*Identification of (Ia) as 2,4-di-*O*-methyl-*L*-arabinose.* 0.06 g of (Ia) in water (0.7 ml) was oxidised with bromine (0.11 ml) for two days at room temperature. The reaction mixture was neutralised with silver carbonate and the acid liberated with hydrogen sulphide. After filtering and concentration, the product was distilled *in vacuo* in order to obtain the lactone, and then treated with methanolic ammonia over night at 0°C. On concentration the amide crystallised and was recrystallised from ethanol. M.p. 160—161°.

0.06 g of (Ia) was boiled with aniline (0.1 ml) in anhydrous ethanol (3 ml) for 4 h. The reaction mixture was concentrated and dried in a desiccator over phosphorus pentoxide. The 2,4-di-*O*-methyl-N-phenylarabinosylamine was recrystallised from butanol, m.p. 142—144°.

These melting points are in good agreement with those of 2,4-di-*O*-methyl-L-arabonamide and 2,4-di-*O*-methyl-N-phenyl-L-arabinosylamine reported by Smith [6] and Jones [1].

*Identification of (Ib) as 2,3,4-tri-*O*-methyl-*L*-arabinose.* 0.11 g of (Ib) was oxidised with bromine and worked up in the same manner as described above. The amide was crystallised from benzene-acetone-ethanol. M.p. 104—105°, undepressed on admixture with an authentic specimen of 2,3,4-tri-*O*-methyl-L-arabonamide.

Methylation of (II) and hydrolysis of the methylated product. The fraction containing (II) was purified on thick filter paper, yielding 0.11 g of a chromatographically pure (II). This was dissolved in water (5 ml) and methylated with methyl sulphate (1 ml) and 30 % sodium hydroxide (2 ml) in the same manner as described above.

The premethylated disaccharide was carefully dried and methylated with silver oxide (2 g) and methyl iodide (2 ml) in dimethyl formamide and worked up as above. The product formed a soft crystalline mass but no attempt of recrystallisation was made.

Hydrolysis of the completely methylated disaccharide was performed in 0.5 N hydrochloric acid (4 ml) at 100°C for 14 h. Neutralisation (IR 4B) and concentration yielded a light coloured syrup (0.11 g).

The methyl ethers from the hydrolysed disaccharide were separated on thick filter paper yielding 0.05 g of a compound with a relatively high R_F-value (IIa) and 0.04 g of a somewhat slower one (IIb).

*Identification of (IIa) as 2,3,4,6-tetra-*O*-methyl-*D*-galactose.* (IIa) was boiled with aniline (0.1 ml) in anhydrous ethanol (3 ml) for 4 h. After concentration and drying over phosphorus pentoxide, the N-phenylgalactosylamine was recrystallised from ethanol. M.p. 199—200°, undepressed on admixture with authentic 2,3,4,6-tetra-*O*-methyl-N-phenyl-D-galactosylamine.

*Identification of (IIb) as 2,3,4-tri-*O*-methyl-*D*-galactose.* The N-phenylgalactosylamine of (IIb) was prepared in the same manner as above. M.p. 167—169°, undepressed on admixture with authentic 2,3,4-tri-*O*-methyl-N-phenyl-D-galactosylamine.

The authors are indebted to Dr. W. M. Hearon, Crown Zellerbach Corp., Camas, Wash., USA for a generous gift of Western larch wood and to *Fonden för Skoglig Forskning* for financial support.

REFERENCES

1. Jones, J. K. N. *J. Chem. Soc.* **1953** 1672.
2. Whistler, R. W. and Smart, C. L. *Polysaccharide Chemistry*, New York 1953, p. 203.
3. Lystad Borgin, G. *J. Am. Chem. Soc.* **71** (1949) 2247.
4. Wadman, W. H., Anderson, A. B. and Hassid, W. Z. *J. Am. Chem. Soc.* **76** (1954) 4097.
5. Pigman, W. W. and Goepp, R. M. Jr. *Chemistry of Carbohydrates*, New York 1948, p. 206.
6. Smith, F. *J. Chem. Soc.* **1939** 744.
7. Kuhn, R., Trischmann, H. and Löw, I. *Angew. Chem.* **67** (1955) 32.

Received August 17, 1956.