344. *Larch ε-Galactan. Part II.* The Isolation of 3-β-L-Arabopyranosyl L-Arabinose.*

By J. K. N. Jones.

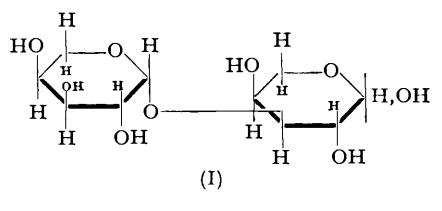
Graded hydrolysis of larch ε-galactan yields a mixture of sugars from which 3-β-L-arabopyranosyl L-arabinose has been isolated and characterised.

L-Arabinose is a common constituent of gums and mucilages and usually occurs, in these substances, in the acid-labile furanose form (cf. Hirst and Jones, *Research*, 1951, **4**, 411). Recently, the pyranose form of L-arabinose has been encountered in Sapote gum (White, *J. Amer. Chem. Soc.*, 1952, **74**, 3966; 1953, **75**, 257). The pyranose form of this sugar is also encountered in the anthocyanins and flavones (*e.g.*, vicianose; Helferich and Bredereck, *Annalen*, 1928, **465**, 166). Previous work (Campbell, Hirst, and Jones, *J.*, 1948, 774; White, *J. Amer. Chem. Soc.*, 1941, **63**, 2871; 1942, **64**, 302, 1507, 2838) had shown that ε-galactan is composed in the main of D-galactopyranose and L-arabofuranose residues, the former predominating. In an attempt to obtain small fragments, the degradation of ε-galactan with hot dilute acid (0·01N) and with cold acid (N) was studied. Examination, at intervals, of the products of hydrolysis on the paper chromatogram showed that arabinose, galactose, and various oligosaccharides were produced. The presence of the latter was demonstrated by spraying the chromatogram with *p*-anisidine hydrochloride and heating it, whereupon several spots, which were either red or brown, appeared. After hydrolysis of the polysaccharide for 1½ hr. at 100°, with 0·01N-hydrochloric acid, the major fraction of the oligosaccharides produced gave the reaction of a pentose and moved more slowly than did galactose. It was isolated by separation of the products of hydrolysis of ε-galactan on columns of cellulose (Hough, Jones, and Wadman, *J.*, 1949, 2511) and identified as 3-β-L-arabopyranosyl L-arabinose (I) on the following evidence.

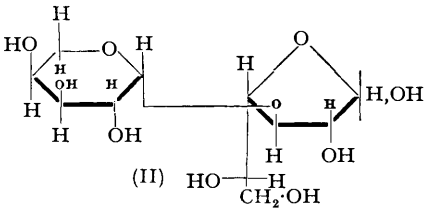
* Part I, *J.*, 1948, 774.

It reduced Fehling's solution, indicating the presence of an aldehyde group, and gave an osazone when heated with phenylhydrazine acetate solution, thus proving the presence of a hydroxyl group on $C_{(2)}$ of the reducing portion of the disaccharide. L-Arabinose was produced on hydrolysis both of the disaccharide and of the osazone. The high positive rotation (+220°) exhibited by (I) in solution indicated that both L-arabinose residues were probably in the pyranose form. On methylation, the disaccharide gave a hexamethyl derivative, which showed an unusually high rotation (+300°); on hydrolysis it gave 2 : 3 : 4-trimethyl and 2 : 4-dimethyl L-arabinose. The trimethyl L-arabinose ($[\alpha]_D$ +120°) was identified by its rate of movement on the chromatogram in butanol–ethanol–water (40 : 11 : 19 by vol.), which is unusually slow for a trimethyl pentose, and as the derived 2 : 3 : 4-trimethyl L-arabonic acid phenylhydrazide. The dimethyl L-arabinose possessed a high positive rotation (+129°) and is therefore a pyranose derivative. This portion of the disaccharide will be methylated on $C_{(2)}$ (see above), and was identified as 2 : 4-dimethyl L-arabinose, since the 2 : 3-dimethyl derivative moves much faster on the paper chromatogram and the 2 : 5-isomer has a negative rotation. This was confirmed by its conversion into the *N*-phenyl-L-arabinosylamine 2 : 4-dimethyl ether (Smith *J.*, 1939, 751) and by its oxidation to a lactone which behaved as a δ-lactone, and therefore possessed a hydroxyl group on $C_{(5)}$. The lactone gave a crystalline phenylhydrazide, but in insufficient amount for characterisation.

The disaccharide probably exists as (II) in the ε-galactan and may have had L-arabofuranose residues attached to the L-arabopyranose portion of the disaccharide. During the formation of the disaccharide (I) by hydrolysis of ε-galactan, it was noted that arabinose and galactose residues were also liberated. This may indicate the presence of galactofuranose residues in ε-galactan. In order to prove that the disaccharide did not arise



(I)



(II)

by resynthesis, a solution of L-arabinose in 0·01N-hydrochloric acid was heated at 100° for 1½ hr. No oligosaccharides were produced.

Gum arabic (Smith, *loc. cit.*), when dissolved in a solution of N-hydrochloric acid and kept for 21 days, was degraded to rhamnose, arabinose, galactose, and oligosaccharides, which were separated by fractionation on cellulose. From the mixture one disaccharide containing arabinose only and a second, very probably 3-α-D-galactopyranosyl L-arabinose, were isolated. The second disaccharide was characterised as its osazone. Derivatives of this disaccharide had been isolated previously and characterised by Smith (*loc. cit.*). Many other gums and mucilages, under these conditions of hydrolysis, yield oligosaccharides containing pentose residues.

As the pairs (*a*) α-D-galactopyranose and β-L-arabopyranose and (*b*) β-D-xylose and β-D-glucuronic acid possess the same configuration of hydroxyl groups on $C_{(1)}$, $C_{(2)}$, $C_{(3)}$, and $C_{(4)}$ and are often associated with one another, it is possible that enzymes can use either the pentose or the hexose as substrate in (*a*) or (*b*) [cf. the presence of 3-D-galactopyranosyl D-galactose in ε-galactan (White, *loc. cit.*) and the synthesis of 3-α-D-glucopyranosyl L-arabinose from D-glucose-1 phosphate, L-arabinose, and sucrose phosphorylase (Doudoroff, Hassid, and Barker, *J. Biol. Chem.*, 1947, **168**, 733)].

Experimental

The following solvents were used in chromatographic separations on Whatman No. 1 paper: (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (*b*) *n*-butanol–pyridine–water (10 : 3 : 3), and (*c*) *n*-butanol–ethanol–water (40 : 11 : 19), all v/v. *p*-Anisidine hydrochloride solution was used as spray to detect sugars. Optical rotations were determined at 20° unless

otherwise stated, and in water. Microanalyses are by Mr. B. S. Noyes of Bristol. Evaporation of solutions was carried out under reduced pressure.

Hydrolysis of ε-Galactan.—Preliminary experiments showed that after 3 weeks ε-galactan (10 g.), dissolved in N-hydrochloric acid (150 c.c.), gave a chromatographic picture [solvent (*b*)] very similar to that obtained after ε-galactan (10 g.) had been heated with 0·01N-hydrochloric acid (70 c.c.) at 100° for 1½ hr. In each case the solution was filtered, neutralised by passage through Amberlite resin IR4B, concentrated, and poured into alcohol. The precipitated, degraded ε-galactan was collected and the filtrate evaporated to a syrupy mixture of sugars (0·7 g.). The syrup was fractionated on a column of cellulose (Hough, Jones, and Wadman, *loc. cit.*), with *n*-butanol half-saturated with water. L-Arabinose appeared first in the effluent, followed by D-galactose, a trace of an unidentified pentose-containing oligosaccharide, 3-β-L-arabopyranosyl L-arabinose (I), and then a hexose-containing disaccharide which gave a brown colour when a sample on paper was heated after spraying. The appropriate fraction of the column effluent containing (I) was collected and concentrated (yield, *ca.* 0·6%).

The material, $[\alpha]_D$ + 220° ± 10° (*c*, 3·4), was chromatographically pure, its rate of movement relative to galactose was 0·79 in solvent (*a*) and 0·69 in solvent (*b*), and, on hydrolysis with N-hydrochloric acid (1 hr.) it gave arabinose only, identified chromatographically. When (I) was heated with phenylhydrazine acetate, an *osazone* was formed (yield 72%), m. p. 235° after recrystallisation from alcohol (Found: C, 57·7; H, 6·0; N, 12·1. $C_{22}H_{28}O_7N_4$ requires C, 57·4; H, 6·1; N, 12·2%).

The disaccharide (0·294 g.) was dissolved in water (5 c.c.) and methylated by the addition of methyl sulphate (1 c.c.), followed by sodium hydroxide solution (2 c.c.; 30%) dropwise, with stirring. When the solution was non-reducing (12 hr.), sodium hydroxide solution (5 c.c.) was added, followed by methyl sulphate (3 c.c.), dropwise. After 24 hr., the solution was heated to destroy sodium methyl sulphate, and the methylated derivative isolated by continuous extraction of the solution with chloroform. Two further treatments, followed by methylation with Purdie's reagents, gave a syrup (0·216 g.), n_D^{15} 1·4710. The product, distilled in a micro-distillation apparatus, had b. p. (bath-temp.) 160—180°/0·05 mm., n_D^{22} 1·4690, $[\alpha]_D$ + 300° (*c*, 1·0) (yield 0·197 g.) (Found: OMe, 48·6. $C_{16}H_{30}O_9$ requires OMe, 50·6%).

The methylated derivative (0·19 g.) was hydrolysed by boiling it in N-hydrochloric acid (20 c.c.): $[\alpha]_D$ +300° (initial value) ⟶ +132° (3 hr., constant value). The solution was cooled, neutralised with silver carbonate, and filtered. Concentration gave a syrup (0·185 g.) which on the paper chromatogram [solvent (*c*)] showed two sugars, which moved more slowly (0·83 and 0·64) than tetramethyl glucose (1·0). The sugars were separated on a sheet of filter paper [solvent (*c*)], and the appropriate sections of paper extracted with acetone. Concentration of the extracts gave 2 : 3 : 4-trimethyl L-arabinose (90 mg.), $[\alpha]_D$ +120° (*c*, 2·0) (Found: OMe, 46·2. Calc. for $C_8H_{16}O_5$: OMe, 48·4%), and 2 : 4-dimethyl L-arabinose (80 mg.), $[\alpha]_D$ +129° ± 4° (*c*, 1·3) (Found: OMe, 34·1. Calc. for $C_7H_{14}O_5$: OMe, 35·3%).

The trimethyl fraction gave an anilide which did not crystallise. A portion (40 mg.) was oxidised with bromine water until the solution was non-reducing. The 2 : 3 : 4-trimethyl L-arabonic acid (32 mg.) was isolated in the usual manner and converted into the phenylhydrazide. The product, after recrystallisation from ethanol, had m. p. 159°, not depressed on admixture with an authentic specimen (Found: N, 9·2; OMe, 30·8. Calc. for $C_{14}H_{22}O_5N_2$: N, 9·4; OMe, 31·2%).

The dimethyl fraction (35 mg.), heated with aniline (20 mg.) in ethanol (2 c.c.), gave in good yield an arabinosylamine derivative, crystallising in plates, m. p. 145—146° after recrystallisation from *n*-butanol (Found: N, 5·6; OMe, 25·1. Calc. for $C_{13}H_{19}O_4N$: N, 5·5; OMe, 24·7%). On oxidation with bromine water, the sugar (35 mg.) gave a lactone, $[\alpha]_D$ +99° (initial value, *c*, 0·85), +88° (1 hr.), +39° (17 hr., constant). Heating it with alcoholic phenylhydrazone gave a crystalline derivative, but in quantity insufficient for analyses.

Hydrolysis of Gum Arabic.—The gum (Turc. variety) (10 g.) was dissolved in N-hydrochloric acid (50 c.c.) and left at room temperature (18° ± 2°) for 21 days. Chromatographic examination of the solution then indicated the presence of arabinose, a trace of galactose, and two oligosaccharides, which moved at speeds (0·41 and 0·33) relative to arabinose (1·0) in solvent (*b*). The solution was filtered and neutralised with Amberlite resin IR4B, and the degraded gum precipitated with alcohol. This material was collected, and the filtrate evaporated to a syrup (2 g.), which was fractionated on a column of cellulose as described above. The effluent was examined chromatographically and the appropriate fractions were collected and concentrated. The major oligosaccharide component was a syrup which showed a rate of movement slower (0·48) than that of galactose (1·0) in solvent (*a*) (yield, 0·11 g.), $[\alpha]_D$ +133° (*c*, 2·1). Heating it

with N-hydrochloric acid for 3 hr. gave an equimolecular mixture of galactose and arabinose. The disaccharide, heated with aqueous phenylhydrazine acetate, gave a *phenylosazone* (in good yield), m. p. 240° after recrystallisation from ethanol (Found: C, 56·2; H, 6·0; N, 11·8. $C_{23}H_{30}O_8N_4$ requires C, 56·4; H, 6·1; N, 11·4%). On hydrolysis of the osazone with boiling N-hydrochloric acid (1 hr.), galactose (detected chromatographically) was liberated.

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