

156. The ϵ -Galactan of Larch Wood (*Larix decidua*).

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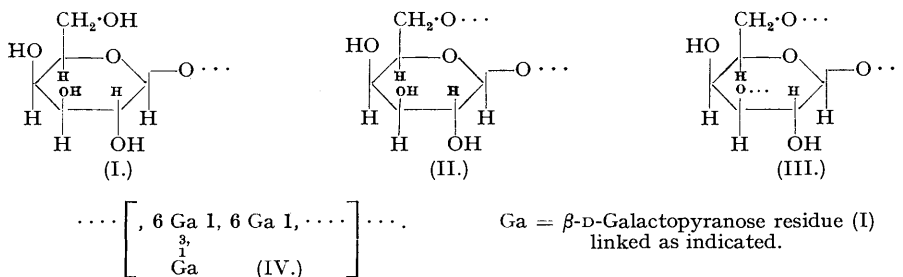
The structure of the ϵ -galactan of European larch wood has been investigated. It is shown that the methylated derivative is a mixture, one portion of which contains at the most only a trace of pentosan, whilst the other is rich in pentosan. The methylated derivative of the pentosan-free portion gave on hydrolysis 2 : 3 : 4 : 6-tetramethyl galactose, 2 : 3 : 4-trimethyl galactose, and 2 : 4-dimethyl galactose in equimolecular proportions. The structural significance of these results is discussed. By graded hydrolysis the pentose residues were removed from the crude ϵ -galactan, and on hydrolysis of the methylated degraded polysaccharide 2 : 4 : 6-trimethyl galactose was obtained in addition to the three sugars mentioned above. It is concluded that ϵ -galactan is a mixture of polysaccharides, one of which is a galactan and the other a galacto-araban.

THE occurrence of the material designated ϵ -galactan, which is of interest in connection with the chemistry of wood formation, was first reported by Trimble (*Amer. J. Pharm.*, 1898, **70**, 152) who showed that it contained galactose residues. Later work (*e.g.*, Schorger and Smith, *Ind. Eng. Chem.*, 1916, **8**, 494; Wise and Peterson, *ibid.*, 1930, **22**, 362; Wise and Unkauf, *Cellulose Chemie*, 1933, **14**, 20; Nikilin and Soloviev, *J. Appl. Chem. Russia*, 1935, **8**, 1016; Owens, *J. Amer. Chem. Soc.*, 1940, **62**, 930; Heusemann, *Naturwiss.*, 1939, **27**, 595) has shown that arabinose is present also, but no clear decision has been reached as to whether the so-called ϵ -galactan is in fact a single polysaccharide of the galacto-araban class or whether it is a mixture of polysaccharides, one of which is a true galactan. The work of Peterson, Barry, Unkauf, and Wise (*loc. cit.*) indicated its heterogeneity, but on the other hand White (*J. Amer. Chem. Soc.*, 1941, **63**, 2871; 1942, **64**, 302, 1507, 2838) interpreted his results as indicating that ϵ -galactan is an arabo-galactan and not a mixture. In our opinion, however, the experimental evidence adduced by White is equally in accord with the view that the crude polysaccharide material is a mixture, since the preparations he used in his investigations were not subjected to rigorous fractionation as a test of homogeneity. On the other hand, in the course of experiments on ϵ -galactan of European larch commenced some years ago (the main conclusions were summarised in *Nature*, 1941, **147**, 25) we found that the polysaccharide could be methylated readily by the thallos ethoxide-methyl iodide method and that the fully methylated derivative was easily separable on fractionation into portions one of which was rich in pentosan whilst the other contained only a trace. These observations, therefore, provide strong evidence in favour of the heterogeneity of the original ϵ -galactan.

Further evidence pointing to the mixed nature of ϵ -galactan was provided by comparative studies of the hydrolysis products resulting respectively from (*a*) that fraction of the methylated crude ϵ -galactan which contained only a trace of pentosan and (*b*) the methylated derivative obtained from the arabinose-free material which is readily prepared by the partial hydrolysis of the crude ϵ -galactan. The arabinose residues, being of the furanose type, are easily hydrolysed with little dislocation of the more resistant galactopyranose residues. Now it has been found that these two methylated derivatives do not yield on hydrolysis the same mixture of partially methylated galactoses. The material (*b*) from the partly hydrolysed polysaccharide yielded, in addition to all the products obtained from (*a*), an additional substance, namely, 2 : 4 : 6-trimethyl galactose. It follows, therefore, that (*b*) [which from its mode of preparation contained a large proportion of (*a*)] contained also a polysaccharide of structure different from that present in (*a*). The position now reached is that the ϵ -galactan we examined was a mixture which contained as one of its components a true galactan. In addition there must be present either a galacto-araban or a mixture of an araban with a galactan differing in structure from the galactan just mentioned.

It is possible to gain from the present results a general picture of the type of structure present in the true galactan portion of the ϵ -galactan. The methylated derivative gave on hydrolysis

the following three sugars in approximately equimolecular proportions: 2:3:4:6-tetramethyl D-galactose, 2:3:4-trimethyl D-galactose, and 2:4-dimethyl D-galactose. It follows that the three D-galactopyranose residues which make up the repeating unit of the galactan molecule are respectively a terminal galactose residue attached only through C₁ (Ga 1 . . .) (I), a residue attached to the others through C₁, C₃, and C₆ ($\therefore \frac{3}{6}$ Ga 1 . . .) (III), and a residue attached



through C₁ and C₆ (. . . 6 Ga 1 . . .) (II). The problem is formally similar to that discussed in connection with the arabans (Hirst, *J.*, 1942, 71; Hirst and Jones, *J.*, 1938, 496; 1939, 452, 454; 1947, 1221), and an example of the general type to which the structure must conform is given in (IV) above, in which a chain of β -linked galactopyranose residues has another β -galactose residue attached as a side chain.

It is clear, however, that the molecular weights of the component polysaccharides of ϵ -galactan must be large since ultracentrifuge measurements have shown that this material is a mixture of two polysaccharides of approximate molecular weight 16,000 and 100,000 respectively (Mosiman and Svedberg, *Kolloid-Z.*, 1942, 100, 99). The figure of 2300 obtained from a study of the viscosity of aqueous solution of ϵ -galactan (Owens, *J. Amer. Chem. Soc.*, 1940, 62, 930) is clearly too low since the constant used for the calculation of the molecular weight of linear polymers of the cellulose type is not applicable to branched-chain substances of the ϵ -galactan structure.

EXPERIMENTAL.

The polysaccharide (for method of preparation see references in the theoretical part) was a pale yellow hygroscopic powder, freely soluble in water giving solutions of low viscosity which gave no colour with aqueous iodine. It gave the following results on analysis: Ash, nil; $[\alpha]_D + 20^\circ$ (approximately) in water; arabinose content (from furfuraldehyde determination), 12.7%; galactose on hydrolysis, 80%; uronic acid content, ca. 2%, iodine number (Bergman and Macherer method) corresponding to repeating unit of ca. 3000 equiv. wt. These figures are in general agreement with values recorded by other workers. Extraction of the polysaccharide (2 g.) with 70% alcohol for 3 months failed to remove any araban.

Hydrolysis of ϵ -Galactan.—The polysaccharide (2.59 g.) was heated with 0.01N-sulphuric acid (50 c.c.) at 90° for 36 hours. The optical rotation of the solution was not observable. The cooled solution was neutralised with barium carbonate and filtered, and the filtrate evaporated to dryness in a partial vacuum at 40°. The residual solid was extracted with methyl alcohol and the extracts concentrated to a syrup which gave, on reaction with diphenylhydrazine, L-arabinose diphenylhydrazone, m. p. 204° (0.32 g.), equivalent to the presence of 8.2% of araban (Found: 12.7% araban from the yield of furfuraldehyde on distillation under standard conditions with 12% hydrochloric acid).

Methylation of ϵ -Galactan.—The galactan (26.5 g.) was methylated (for details see Hirst and Jones, *J.*, 1938, 496) by boiling the thallose hydroxide complex with methyl iodide and the product (20.5 g.) (Found: OMe, 42.9%) fractionated from acetone solution by the portionwise addition of light petroleum (b. p. 60–80°) yielding: Fraction A (13 g.), $[\alpha]_D^{19} - 18^\circ$ (c, 0.81 in methyl alcohol) (Found: OMe, 42.5; furfuraldehyde, on boiling with 12% hydrochloric acid, less than 2%). Fraction B (4 g.), $[\alpha]_D^{19} - 19^\circ$ (c, 0.96 in methyl alcohol) (Found: OMe, 42.4; furfuraldehyde, 4.7%, equivalent to approximately 12% of dimethyl araban). Fraction C (3 g.), $[\alpha]_D^{19} - 16^\circ$ (c, 1.22 in methyl alcohol) (Found: OMe, 44.3%). Fraction D (0.3 g.), a sticky solid which was not further examined.

Hydrolysis of Methylated Galactan.—Fraction A (13 g.) was dissolved in methyl-alcoholic hydrogen chloride (150 c.c.; 8%) and the solution boiled under reflux for 60 hours. The solution was cooled, neutralised with silver carbonate, and filtered, and the filtrate concentrated under reduced pressure to a syrup (14.45 g., $n_D^{20} 1.4652$) which was distilled under reduced pressure and the following fractions collected: Fraction 1 (2.78 g.), b. p. 106–110°/0.01 mm., $n_D^{18} 1.4462$ (Found: OMe, 60.5%). Fraction 2 (2.83 g.), b. p. 114–133°/0.01 mm., $n_D^{17} 1.4517$ (Found: OMe, 59%). Fraction 3 (3.29 g.), b. p. 133–142°/0.01 mm., $n_D^{17} 1.4630$ (Found: OMe, 51%). Fraction 4 (1.28 g.), b. p. 120–140°/0.001 mm., $n_D^{18} 1.4748$ (Found: OMe, 43%). Fraction 5 (1.63 g.), b. p. 140–145°/0.001 mm., $n_D^{18} 1.4755$ (Found: OMe, 41.9%). Fraction 6 (1.18 g.), b. p. 145–160°/0.01 mm., $n_D^{19} 1.4842$ (Found: OMe, 38.4%). The still residue (1.3 g.) was not further examined.

Examination of the Fractions.—Fractions 1 and 2, because of their similar constants, were combined and hydrolysed with boiling N-hydrochloric acid (100 c.c.), $[\alpha]_D^{18} + 82^\circ$ (initial value); + 90° (1½ hours); + 86° (2 hours, constant value). The free sugar was isolated in the usual manner and the product (5.2 g.) fractionally distilled in a vacuum giving Fractions 7 to 9. Fraction 7 (2.2 g.) had

b. p. 112—118° (bath temp.)/0.001 mm., n_D^{18} 1.4605, $[\alpha]_D^{20} + 88^\circ$ (c, 0.49 in water) (Found: OMe, 50%). On being heated with alcoholic aniline this fraction gave 2:3:4:6-tetramethyl D-galactose anilide, m. p. 202°. The low rotation of the syrup comprising Fraction 7 indicated that some impurity was present. The yield of tetramethyl galactose anilide showed, however, that only small amounts of foreign material were present. No trimethyl arabinose was detectable, although any of the substances arising from the pentosan responsible for the trace of furfuraldehyde material above would be found in this particular fraction. Even if all the impurity were calculated as trimethyl arabinose its amount (approximately 0.2 g.) would not be significant for our present purpose in comparison with the total amount of tetramethyl galactose (4.1 g.). Fraction 8 (2.07 g.) had b. p. 125—130°/0.001 mm., n_D^{18} 1.4658, $[\alpha]_D^{20} + 99^\circ$ (c, 0.55 in water) (Found: OMe, 49.2%). This fraction gave 2:3:4:6-tetramethyl D-galactose anilide, m. p. 202°, on being boiled with alcoholic aniline. Fraction 9 (0.71 g.) had b. p. 130—170°/0.01 mm., n_D^{18} 1.4715, $[\alpha]_D^{20} + 80^\circ$ (c, 0.41 in water) (Found: OMe, 44.2%). This fraction on being boiled with alcoholic aniline gave a mixture of 2:3:4:6-tetramethyl D-galactose anilide, m. p. 201°, and 2:3:4-trimethyl D-galactose anilide, m. p. 169° (McCreath and Smith, *J.*, 1939, 390), separated by repeated recrystallisation from ethyl alcohol. It is calculated that this fraction contained about 0.2 g. of tetramethyl D-galactose and 0.51 g. of trimethyl D-galactose. Fraction 3 (3.28 g.) was hydrolysed with boiling N-hydrochloric acid (50 c.c.). $[\alpha]_D^{20} + 96^\circ$ (initial value) $\longrightarrow + 89^\circ$ (constant value, 2½ hours). The cooled solution was neutralised with silver carbonate, filtered, and concentrated under reduced pressure to a syrup. A crystalline silver salt (0.2 g.) of an acid separated at this stage and was removed. The filtrate was concentrated and extracted with ether yielding an ether-soluble fraction (2.6 g.), n_D^{19} 1.4760, $[\alpha]_D^{20} + 90^\circ$ (in water) (Found: OMe, 39.8%), and an ether-insoluble fraction (0.3 g.) (Found: OMe, 34%). The ether-soluble fraction on being heated with alcoholic aniline gave 2:3:4-trimethyl D-galactose anilide, m. p. and mixed m. p. with an authentic sample 169°. No other crystalline anilide could be isolated.

The ether-insoluble fraction on being boiled with alcoholic aniline gave 2:4-dimethyl D-galactose anilide, m. p. and mixed m. p. with an authentic specimen 211°.

Fractions 4 and 5 were combined (2.87 g.) and hydrolysed with boiling N-hydrochloric acid (50 c.c.). $[\alpha]_D^{20}$ (initial value not observable) $+ 102^\circ$ (½ hour); $+ 96^\circ$ (1 hour); $+ 90^\circ$ (2 hours, constant value). The free sugar (2.6 g.) was isolated in the usual manner and crystallised on standing. The 2:4-dimethyl D-galactose hydrate (0.8 g.) was separated by trituration with acetone followed by filtration; m. p. and mixed m. p. with an authentic specimen 104—105°. The non-crystalline sugars on being heated with alcoholic aniline gave 2:4-dimethyl D-galactose anilide, m. p. 207° (decomp.) (Smith, *J.*, 1939, 1734). No other crystalline anilide could be detected. Fraction 6 (1.1 g.) was hydrolysed by heating it with N-hydrochloric acid (50 c.c.), and the resultant sugar (0.9 g.) was isolated in the usual manner (Found: OMe, 31%). On being heated with alcoholic aniline this fraction gave 2:4-dimethyl D-galactose anilide, m. p. 208° (decomp.); no other anilide could be isolated.

Preferential Removal of L-Arabinose from ϵ -Galactan.—As a result of a series of experiments the following conditions were selected for the partial hydrolysis.

The polysaccharide (19.5 g.) was dissolved in 0.01N-sulphuric acid (200 c.c.) and heated at 90° for 40 hours. The solution was then neutralised with barium carbonate and filtered, and the filtrate concentrated under reduced pressure to a syrup. This syrup was then fractionated from aqueous solution by the addition of alcohol and the following fractions were collected:

Fraction I (3 g.), a brown solid (Found: OMe 1.8; furfuraldehyde 2.9%, equivalent to 5.9% araban).

Fraction II (9 g.), a white solid, $[\alpha]_D^{18} + 18^\circ$ (c, 1.64 in water) (Found: Furfuraldehyde 1.4%, equivalent to 2.9% of araban; *M*, from iodine number, 3400).

Fraction III (3.6 g.), a white solid, $[\alpha]_D^{18} + 20^\circ$ (c, 1.94 in water) (Found: Furfuraldehyde, trace; *M*, from iodine number, 3500).

The filtrate from Fraction III was concentrated to a syrup (2.5 g.), made up to 500 c.c. with water, and filtered. The sugar in solution was mainly L-arabinose, $[\alpha]_D^{19} + 98^\circ$ (c, 1.08 in water), and gave, with diphenylhydrazine, L-arabinose diphenylhydrazone, m. p. 205°. The yield of L-arabinose corresponds to the removal of some 9% of pentosan from the ϵ -galactan.

Fraction II (1.04 g.) was then hydrolysed with boiling N-sulphuric acid for 4 hours. The solution was neutralised with barium carbonate, filtered, and concentrated to a syrup which crystallised. Trituration with alcohol gave crystalline D-galactose in 85% yield; no other sugar could be detected.

Methylation of Fractions II and III (Material of Low Pentosan Content).—Fractions II (7 g.) and III (3.0 g., above) were combined and methylated with thallium hydroxide and methyl iodide and the product (9.7 g.) (Found: OMe, 42.4%) fractionated from acetone by the addition of ether, giving: Fraction (a) (2.3 g.), $[\alpha]_D^{20} - 11^\circ$ (c, 1.05 in methyl alcohol) (Found: OMe, 42.0%). Fraction (b) (6.3 g.), $[\alpha]_D^{18} - 12^\circ$ (c, 1.09 in methyl alcohol) (Found: OMe, 42.0%). The residue (1.1 g.) was a syrup and was not further examined.

Fractions (a) and (b) were combined (7.5 g.) and hydrolysed with boiling methyl-alcoholic hydrogen chloride (1.8%; 250 c.c.) for 12 hours. The products of methanolysis (8.37 g.; n_D^{21} 1.4662) were isolated in the usual manner and fractionated in a vacuum, giving: Fraction 10 (2.06 g.), b. p. 100—112°/0.1 mm., n_D^{18} 1.4492 (Found: OMe, 61%). Fraction 11 (1.07 g.), b. p. 115—120°/0.1 mm., n_D^{19} 1.4588 (Found: OMe, 54%). Fraction 12 (0.96 g.), b. p. 140—150°/0.1 mm., n_D^{21} 1.4645 (Found: OMe, 50.7%). Fraction 13 (2.79 g.), b. p. 140°/0.1 mm., n_D^{22} 1.4756 (Found: OMe, 43%).

Fraction 10 on hydrolysis with boiling N-hydrochloric acid for 4 hours gave 2:3:4:6-tetramethyl D-galactose, $[\alpha]_D^{20} + 100^\circ$ (c, 0.98 in water), which with alcoholic aniline gave 2:3:4:6-tetramethyl D-galactose anilide, m. p. 200°.

Fractions 11 and 12 were combined and the whole (2.0 g.) hydrolysed with N-hydrochloric acid (50 c.c.) on the boiling water-bath for 4 hours. The sugar isolated in the usual manner showed $[\alpha]_D^{19} + 84^\circ$ (c, 0.55 in water) (Found: OMe, 40.5%), and on being heated with alcoholic aniline gave a mixture of the anilides of 2:3:4- and 2:4:6-trimethyl D-galactose, m. p. 169° (McCreath and Smith, *loc. cit.*) and 179° (Percival, *J.*, 1937, 1615) respectively. The separation and identification of these

two anilides was aided by their characteristic crystalline shapes (plates and needles respectively). It was not possible to estimate quantitatively the relative proportions of each sugar.

Fraction 13 (2.76 g.) on hydrolysis gave 2:4-dimethyl D-galactose which crystallised as the monohydrate, m. p. 104°. The non-crystalline residues on being heated with alcoholic aniline gave 2:4-dimethyl D-galactose anilide, m. p. 210° after recrystallisation from water. No other sugar could be detected.

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