These data indicate that this substance is the lower rotating 1-epimer of 1-(9-adenyl)-1-deoxy-1-*O*-methyl-*aldehydo*-D-galactose aldehydrol (IX), herein designated as the Second Form.

9-(Tetra-O-acetyl-β-D-galactopyranosyl)adenine picrate. A mixture of 28.0 g. of 6-acetamido-9-chloromercuripurine (V),[9] 28.0 g. of cadmium carbonate, 5 g. of Celite,[10] and 425 ml. of xylene was dried azeotropically by distillation of 100 ml. of the xylene. To this mixture was added, at 90° and with stirring, a solution of 28.0 g. of tetra-*O*-acetyl-α-D-galactopyranosyl bromide[17] in 30 ml. of xylene. The mixture was refluxed for 3 hr., essentially according to the procedure of Davoll and Lowy,[7] filtered hot, and the filtrate was concentrated under reduced pressure. The filter cake and residue were extracted with hot chloroform, the chloroform solution was washed with 30% aqueous potassium iodide, water, dried with anhydrous sodium sulfate, and concentrated under reduced pressure to a sirup; yield 24.0 g. of crude 6-acetamido-9-(tetra-*O*-acetyl-β-D-galactopyranosyl)purine. The crude product (13.1 g.) was converted to the picrate by boiling for 1 min. with 67 ml. of 10% picric acid in ethanol.[8] The picrate of 9-(tetra-*O*-acetyl-β-D-galactopyranosyl)adenine readily crystallized upon cooling; yield 12.4 g. (63%). The material was further purified by column chromatography[11] by placing 0.8-g. portions of material on (100 × 44 mm.) columns of Micro-Cel C[10] using 150 ml. of benzene-ethanol (5:1 by vol.) as developer. The material in the bright yellow zone was eluted with acetone and crystallized from ethanol as deep yellow needles, dec. 284–287°.

Anal. Calcd. for $C_{25}H_{26}N_8O_{16}$: N, 16.14. Found: N, 15.90.

9-(Tetra-O-acetyl-β-D-galactopyranosyl)adenine. A mixture of 9-(tetra-*O*-acetyl-β-D-galactopyranosyl)adenine picrate (7.4 g.) was converted to the nucleoside by treatment with Dowex-1 as described above for the acyclic derivative. The resulting sirup (3.7 g., 75%) was concentrated under reduced pressure twice from ethanol, dissolved in 25 ml. of ethanol, and allowed to evaporate from an open beaker at room temperature. Crystallization occurred in 24 hr. The material was filtered and washed with cold methanol; yield 1.13 g. (26%) of 9-(tetra-*O*-acetyl-β-D-galactopyranosyl)adenine. Recrystallization from methanol produced fine white needles; m.p. 212–213.5°, $[\alpha]_D^{24}$ +7.3° (*c*, 2.6 chloroform); absorption spectra data[13]: $\lambda_{max}^{C_2H_5OH}$ 248 mμ, λ_{max}^{KBr} 2.88, 3.05 μ (NH, NH_2), 5.65 μ (C=O of acetates), 5.95, 6.15, 6.70, 6.96 μ (NH_2 and purine ring), 7.28 μ (methyl hydrogens), 8.05–8.22, 9.15, 9.45 μ (C—O—C); x-ray powder diffraction data[14]: 11.07 vs (1), 9.72 vw, 8.69 vw, 7.86 m, 6.76 m, 6.38 w, 6.04 w, 5.65 m, 5.10 s (2), 4.82 m (3), 4.31 m, 4.19 m, 3.94 w, 3.85 vw.

Anal. Calcd. for $C_{19}H_{23}N_5O_9$: C, 49.02; H, 4.98; N, 15.15. Found: C, 48.95; H, 4.40; N, 15.52.

9-β-D-Galactopyranosyladenine. A solution of 24.0 g. of crude 6-acetamido-9-(tetra-*O*-acetyl-β-D-galactopyranosyl)-purine in 225 ml. of dry methanol and 35 ml. of *n*-butylamine was refluxed for 5 hr. The solution was concentrated to 50 ml. under reduced pressure and held at 0° until precipitation occurred. The precipitate was filtered and washed with cold methanol, chloroform, and ether; yield 9.6 g. (67.5%). The solid was dissolved in water, decolorized with carbon and concentrated to a sirup which crystallized. The mass was triturated with methanol, filtered, and dried; m.p. 192–195°. Further purification was effected by treatment with hot 10% ethanolic picric acid to form a crystalline 9-(β-D-galactopyranosyl)adenine picrate; m.p. 230–232°.

Anal. Calcd. for $C_{17}H_{18}N_8O_{12}$: C, 38.79; H, 3.45; N, 21.29. Found: C, 38.83; H, 3.65; N, 19.98.

Regeneration of the nucleoside from the picrate with Dowex-1 (carbonate form)[12] anion exchange resin by the method described above for the acyclic picrate derivative gave fine colorless needles, m.p. 198–200°, $[\alpha]_D^{23}$ +95.5° (*c*, 0.5 water); absorption spectra data[13]: $\lambda_{max}^{H_2O}$ 260 mμ; λ_{max}^{KBr} 2.90, 2.98 μ (OH, NH), 6.02, 6.24, 6.32, 6.78 μ (NH_2, NH, and purine ring), 9.20–9.50, 10.0 μ (C—O—C, C—OH); x-ray powder diffraction data[14]: 7.65 w, 7.00 vw, 6.59 m, 6.35 m, 5.88 w, 5.20 vw, 4.88 vs (1), 4.60 w, 4.32 vw, 4.18 w, 4.08 vw, 3.97 w, 3.60 m, 3.50 m (3), 3.42 w, 3.36 w, 3.26 s, (2), 3.17 w, 3.07 vw, 2.94 w, 2.89 w.

Anal. Calcd. for $C_{11}H_{15}N_5O_5$: C, 44.43; H, 5.08; N, 23.54. Found: C, 43.85; H, 5.36; N, 23.31.

Identical material was also obtained on deacetylation, with boiling methanolic *n*-butylamine, of 9-(tetra-*O*-acetyl-β-D-galactopyranosyl)adenine by the same procedure used to deacetylate VIII.

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[17] H. Ohle, W. Marecek, and W. Bourjau, *Ber.*, **62**, 833 (1929).

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, HAWAII AGRICULTURAL EXPERIMENT STATION
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The Constitution of a Galactomannan from the Seed of *Leucaena glauca*[1]

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A galactomannan, composed of 57% mannose and 43% galactose, was isolated in 25% yield from the seed of *Leucaena glauca.* The polysaccharide had an average D.P. of 150 and gave highly viscous aqueous solutions at low solute concentrations. Periodate degradation revealed that some mannose residues and a smaller number of galactose residues were not attacked. Hydrolysis of the methylated gum showed that galactose occupied mainly terminal, nonreducing positions and the considerable quantity of 2,3,4,6-tetra-*O*-methyl-D-mannose found indicated the presence of some major branch-points. Further evidence that some 1,3-linkages were present was obtained.

DISCUSSION

The seed of *Leucaena glauca* (better known as koa hoale in the Hawaiian Islands) has an interesting chemical composition. Among the nitrogenous constituents found in the seed, a poisonous amino acid known as mimosine[3] is present in relatively large quantities. The isolation of this amino acid which

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[3] J. Renz, *Z. Physiol. Chem.*, **244**, 153 (1936); H. Nienburg and K. Taubock, *Z. Physiol. Chem.*, **250**, 80 (1937); D. Kostermans, *Rev. Trav. Chim.*, **65**, 319 (1946).

contains a 3-hydroxypyridone group is made somewhat difficult due to the presence of a galactomannan in considerable amounts. Thus it was found that up to 25% yields of the galactomannan could be obtained from the dry and coarsely ground seed by hot water extraction.[4] Precipitation of the gum in alcohol gave the usual feathery and fibrous precipitate so often encountered in the isolation[4,5] of galactomannans.

The physical and chemical properties of plant gums and mucilages has been fully discussed in the recent monograph by Smith and Montgomery.[5] In view of this, some of the general structural aspects of galactomannans from such sources as guar and locust bean seeds need not be reviewed here.

The galactomannan from the seed of *Leucaena glauca* was found to contain a somewhat greater proportion of galactose (43%) than that found in guar gum. The final product obtained after a mild alkali treatment to remove any associated protein possessed an average D.P. of only 150. The product still gave, however, highly viscous solutions. The relative viscosity of 0.1% solutions of the polysaccharide was 2.52 at 20°.

Periodate degradation of the galactomannan employing the powerful modification of Smith and associates[6] revealed that about 15 to 17% of the hexose residues (mainly mannose) were not attacked. A smaller quantity of galactose residues survived the periodate treatment. Hydrolysis of the nonreducing component which had a lesser R_f value than glucose or galactose in a number of different solvent systems gave mannose and erythritol in major quantities and galactose and glycerol in minor amounts. This observation indicated that some unusual linkages were present and that a small percentage of galactose residues apparently occupied inter-chain positions. The release of glycerol upon the complete acid hydrolysis of the nonreducing component indicated that a few 1,6-linkages were present.

The relatively large amounts of glycerol and erythritol released upon mild acid hydrolysis (0.25*N* sulfuric, 20°) of the polyalcohol indicated that the main polymeric linkage was of the 1,4-type. The galactose residues were apparently present predominantly as terminal, non-reducing single branch units in the manner found for instance in gum guar.[7-9] The amount of periodate consumed (1.18 moles) per mole of hexose and the large amount of formic acid formed (0.75 mole per hexose unit) further indicated the presence of numerous terminal, nonreducing residues.

Further information concerning the structure of the gum was forthcoming from methylation studies. The large molar quantity (36% of total methylated sugars) of 2,3,4,6-tetra-*O*-methylated-D-galactose showed that the majority of the galactose residues were indeed situated as terminal, nonreducing units. The presence of a methylated galactose residue in the fraction first believed to consist only of 2,3,6-tri-*O*-methyl-D-mannose indicated that some galactose units did in fact occupy interchain positions. This was in agreement with the results from the periodate oxidations. It is believed that the majority of the methylated galactose found with the 2,3,6-tri-*O*-methyl-D-mannose fraction is 2,3,6-tri-*O*-methyl with a lesser quantity of 2,4,6-tri-*O*-methyl-D-galactose. The latter residue would be resistant to periodate attack in the original polysaccharide as was observed in the periodate degradation studies.

The 1:13.5 molar ratio of 2,3,4,6-tetra-*O*-methyl-D-mannose to the combined total of all other components indicated that a major branch point was present at about every seventh or eighth mannose residue along the main chain. Based on a D.P. of about 150, then each molecule contained about 10 to 12 major branch points. The ratio (1.3:1) of 2,3,4,6-tetra-*O*-methyl-D-galactose plus 2,3,4,6-tetra-*O*-methyl-D-mannose to 2,3-di-*O*-methyl-D-mannose further indicated that major branch points were present along the main 1,4-linked mannose chain. If no major branch points had been present, the above ratio should have approached a 1:1 value.

The isolation of about 11% 2,4,6-tri-*O*-methyl-D-mannose showed that some of the mannose residues were joined in the original polysaccharides by 1,3-linkages. This observation was in agreement with the results obtained in the periodate degradation studies. The quantity of this trimethylmannose derivative was somewhat lower than the total amount of hexose not attacked by periodate. It is believed that this difference was due to the presence of the as yet unidentified trimethylgalactose derivatives mentioned earlier, namely, 2,4,6- and 2,3,6-tri-*O*-methyl-D-galactose.

In summary, the results obtained from the periodate degradation and methylation studies indicated that the main structural features of the galactomannan from *Leucaena glauca* seed were similar to those of other legume seed galactomannans. The relatively high yield of 2,3,4,6-tetra-*O*-methyl-D-mannose obtained upon hydrolysis of the methylated polysaccharide indicated that major branch-points were apparently present in the molecule. In this respect, the structure of the gum may be some-

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(4) E. Anderson, *Ind. Eng. Chem.*, **41**, 2887 (1949).

(5) F. Smith and R. Montgomery, *Chemistry of Plant Gums and Mucilages* A.C.S. Monograph No. 141, Reinhold, New York, 1959.

(6) I. J. Goldstein, G. W. Hay, Bertha A. Lewis, and F. Smith, Abstr. 135th Am. Chem. Soc. Meeting, Boston, Mass., April 1959, p. 3D.

(7) C. M. Rafique and F. Smith, *J. Am. Chem. Soc.*, **72**, 4634 (1950).

(8) J. W. Swanson, *J. Am. Chem. Soc.*, **71**, 1510 (1959).

(9) Z. F. Ahmed and R. L. Whistler, *J. Am. Chem. Soc.*, **72**, 2524 (1950).

what similar to that proposed[5,10] for Kentucky coffee bean gum. The presence of small numbers of unusual linkages[5] was established to some extent. Both periodate degradation and methylation results showed that some 1,3-linkages involving mannose were present. Evidence was obtained to the effect that some galactose residues were resistant to attack by periodate and that they occupied interchain positions.

EXPERIMENTAL

The solvent systems used for the paper chromatographic resolution of the sugars were: A, *n*-butyl alcohol:pyridine: water (6:4:3)[11]; B, *n*-butyl alcohol:ethanol:water (4:1:5)[12]; C, *n*-butyl alcohol:acetic acid:water (4:1:3)[12]; and D, butanone:water azeotrope.[13] The spray reagents used for the detection of the compounds on the chromatograms were E, *p*-anisidine trichloroacetate[14] and F, ammoniacal silver nitrate solution.[15] All evaporations were carried out under reduced pressure and usually with the bath temperature at 35°. The melting points of crystalline compounds were determined on a hostage apparatus and are uncorrected.

Isolation of the galactomannan. Seed from *leucaena glauca* was fed through a Wiley mill which was not fitted with a screen. After several repetitions of this operation, practically all of the seeds were sliced several times. The split seeds were extracted with hot water until virtually no further precipitate was obtained by adding the extract to ethanol. The combined extract was filtered while hot and the filtrate added with vigorous stirring to three volumes of ethanol. The feathery precipitate was collected, redissolved in 10 % sodium hydroxide and the solution heated to near boiling for about 2 hr. to decompose any associated protein. The solution was cooled, acidified with acetic acid and the polysaccharide precipitated in alcohol as before. The product was redissolved and precipitated until a nearly white product was obtained. The yield of the galactomannan from the seed was 24 to 25 %.

Composition of the galactomannan. A portion (1.50 g.) was suspended in *N* sulfuric acid (25 ml.) and the contents refluxed for 8 hr. The solution was neutralized (barium carbonate), decolorized with charcoal, and filtered while hot. The filtrate was evaporated and the sirupy residue chromatographed using solvents A, B, and C and the developed chromatograms sprayed with reagent E. Components corresponding to mannose and galactose were observed. The molar ratio of the two components was determined by the phenol-sulfuric acid method[16] and found to be 43% galactose and 57% mannose.

Identification of the sugars. Part of the above hydrolysis mixture (1.0 g.) was resolved by sheet paper (Whatman 3MM paper) chromatography using solvent A. The two sugars were located by excision of guide strips and spraying these with reagent E. The two hexoses were eluted with water, the eluates concentrated and the compounds identified as follows:

(a) D-*Mannose.* The syrupy product from above was induced to crystallize by the addition of a few seed crystals of D-mannose. After recrystallization from a water-ethanol mixture, the isolated D-mannose had m.p. and mixed m.p. 132°, $[\alpha]_D^{25}$ +14.1° equilibrium value in water (*c*, 3.5). To an aqueous solution of the sugar was added phenylhydrazine hydrochloride (about 5% molar excess) and sodium acetate. The mixture was warmed slightly and then allowed to stand at room temperature for 3 to 4 hr. after which time the crystalline hydrazone was collected by filtration. The product was washed with cold ethanol and ether and after drying in a vacuum desiccator, the mannose phenylhydrazone had m.p. and mixed m.p. 198–199°, $[\alpha]_D^{25}$ +33° in pyridine (*c*, 2.5).

(b) D-*Galactose.* The sirupy product was induced to crystallize after seeding. Recrystallization of a portion from an ethanol-water mixture gave D-galactose, m.p. and mixed m.p. 166°, $[\alpha]_D^{25}$ +80° equilibrium value in water (*c*, 2.0). Part of the residue was dissolved in a 1:1 mixture of water and concentrated nitric acid and the solution heated on a boiling water bath for 2 to 3 hr. The solution was reduced to a small volume after which ice water was added. Crystalline mucic acid separated which after washing with ethanol and drying had m.p. and mixed m.p. 218°.

Viscosity of aqueous solutions of the gum. The relative viscosity of a 0.1% solution of the gum was determined at 20° using an Ostwald viscometer. The relative viscosity of the gum solution was found to be 2.52. Under similar conditions, guar gum was found to have a relative viscosity of 2.95 while that of gum arabic was 1.23.

Determination of the D.P. The average D.P. of the gum was determined by a chemical procedure.[17] The galactomannan (250 mg.) was suspended in a solution (20 ml.) of potassium borohydride (300 mg.).[18] The reduction was allowed to proceed at room temperature for 48 hr. after which time the solution was acidified with acetic acid. The solution was cooled (5°) and periodic acid added (about 25% molar excess) and the final volume adjusted to 35 ml. The reaction mixture was kept in the dark at 5° and periodically aliquots (3 ml.) were withdrawn to determine formaldehyde. The technique for the determination of formaldehyde in the presence of the galactomannan polyaldehyde is described elsewhere.[17] No further formaldehyde was evolved after 72 hr. standing and at this time, the average D.P. of the galactomannan was found to be 150.

$$\text{D.P.} = \frac{\text{Wt. of sample} \times 30 \times 2}{\text{Wt. of CH}_2\text{O} \times 162}$$

Periodate degradation of the gum. A quantity (1.50 g.) of the gum was suspended in water and 13 mmoles of periodic acid added. The volume of the reaction mixture was adjusted to 200 ml. and the mixture left at room temperature and in the dark. Periodically, periodate consumption was determined by the usual iodometric titration. After about 70 hr., no further periodate consumption could be detected and at this time, 1.18 moles of periodate had been consumed per mole of hexose. Formic acid was determined by titration using an automatic titrimeter and after about 90 hr. 0.75 mole of formic acid had been produced per hexose residue. Little or no change was observed thereafter. The reaction mixture was treated with barium carbonate and filtered. To the filtrate was added potassium borohydride (1.2 g.) to effect reduction of the polyaldehyde to the corresponding polyalcohol.[19] After standing at room temperature for 24 hr., the solution was carefully neutralized with dilute hydrochloric

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(10) E. B. Larson and F. Smith, *J. Am. Chem. Soc.*, **79**, 429 (1955).

(11) A. Jeanes, C. S. Wise, and R. J. Dimler, *Anal. Chem.*, **23**, 415 (1951).

(12) S. M. Partridge and R. G. Westall, *Biochem. J.*, **42**, 238 (1948).

(13) L. Boggs, L. S. Cuendet, I. Ehrenthal, R. Koch, and F. Smith, *Nature*, **166**, 520 (1950).

(14) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.* **1702** (1950); L. Mukherjee and H. C. Srivastava, *Nature*, **169**, 330 (1952).

(15) S. M. Partridge, *Nature*, **158**, 270 (1946).

(16) M. Dubois, J. K. Hamilton, K. A. Gilles, P. Rebers, and F. Smith, *Anal. Chem.*, **28**, 250 (1956).

(17) A. M. Unrau and F. Smith, *Chem. & Ind.*, 330 (1957).

(18) M. Abdel-Akher, J. K. Hamilton, and F. Smith, *J. Am. Chem. Soc.*, **73**, 4691 (1951).

(19) F. Smith and J. W. Van Cleve, *J. Am. Chem. Soc.*, **77**, 3091 (1955); M. Abdel-Akher, J. E. Cadott, Bertha A. Lewis, R. Montgomery, F. Smith, and J. W. Van Cleve, *Nature*, **171**, 474 (1953); M. Abdel-Akher, J. K. Hamilton and F. Smith, *J. Am. Chem. Soc.*, **73**, 4691 (1951).

acid. The solution was evaporated (bath temperature 25–27°) and the residue treated several times with methanol to affect removal of borate.[20] Finally, the residue was dissolved in cold 0.25*N* sulfuric acid (25 ml.) and the solution left at room temperature for about 14 hr. This mild hydrolysis treatment affects cleavage of the true acetal systems whereas the glycosidic bonds are left intact.[6] The solution was deionized and the residue examined chromatographically. Using solvents A, B and C and spray reagents E and F, large quantities of glycerol and erythritol were detected. A nonreducing component which had a smaller R_f value than glucose or galactose was also evident. The components were resolved using solvent A and Whatman 3MM paper. The relative quantities of glycerol and erythritol were determined by the periodate–chromotropic acid method.[17,21]

(a) *Identification of glycerol.* The oily residue was dried under vacuum (bath temperature 75°) and then treated with pyridine (5 ml.) and a 10% molar excess of *p*-nitrobenzoyl chloride. The mixture was heated for 40 min. at 70° after which time the solution was poured into ice cold saturated sodium bicarbonate. The insoluble product that formed was collected by filtration, washed with water and after recrystallization from acetone, the glycerol tri-*p*-nitrobenzoate had m.p. and mixed m.p. 186–188°.

(b) *Identification of erythritol.* A solution of the sirupy residue was treated with charcoal and the filtrate evaporated to a thick sirup. After standing for 48 hr., the crystals of erythritol which had formed were washed with cold ethanol and after drying they had m.p. and mixed m.p. 117–118°.

(c) *Hydrolysis of the non-reducing component.* After elution of the component from the chromatograms and evaporation of the eluate, the residue was dissolved in *N* sulfuric acid and the solution refluxed for 8 hr. The solution was neutralized (barium carbonate), filtered and evaporated and the product chromatographed. Mannose and erythritol were observed in major amounts. Smaller quantities of galactose and glycerol were also detected. The hydrolyzate was resolved by sheet paper chromatography using solvent A and Whatman 3MM paper. Mannose was converted to its phenylhydrazone, m.p. and mixed m.p. 198–199°. Erythritol was obtained crystalline, m.p. and mixed m.p. 118°. The relative quantities of etythritol and glycerol were determined by the periodate–chromotropic acid method.[17] Mannose and galactose were determined as before by the phenol-sulfuric acid method.[16] The ratio of hexose (mannose plus galactose) to erythritol plus glycerol was found to be 1:1.

The total recovery or yield of components from a complete hydrolysis of the polyalcohol was about 96% based on the moles of hexose in the starting material. The ratio of components in the polyalcohol was found to be as follows: D-Mannose, 13.3%; D-Galactose, 1.8%; Erythritol, 30.5%; Glycerol, 54.2%. In the value for glycerol is included the small quantity (2.4%) found in the hydrolyzate of the nonreducing component described above.

Methylation of the gum. A quantity of gum (2.2 g.) was subjected four times to the usual Haworth methylating reagents[22] followed by three methylations by a procedure described by Kuhn and associates.[23] The final product was a yellow, brittle glass, 2.15 g., yield 78%. The methylated polysaccharide was dissolved in methanol (30 ml.) containing about 1% hydrogen chloride and the solution refluxed for 24 hr. after which time the solvent was evaporated and the product hydrolyzed (0.5*N* hydrochloric acid) for about 12 hr. Most (1.9 g.) of the mixture of methylated sugars was resolved into its individual components using Whatman 3MM paper and solvent D. Where overlapping was evident, the respective components were rechromatographed until each individual component appeared to be essentially homogeneous.

Identification of the components. (a) *Component 1,* R_f 0.78–0.79 in solvent D, 116 mg. The sirup after drying had $[\alpha]_D^{25}$ +2.3° in water (*c*, 2.5). Demethylation of a portion (30 mg.) with 45 % hydrobromic acid gave only one hexose component which chromatographically corresponded to mannose. Other partial demethylation products were also evident. Treatment of the methylated sugar with aniline gave crystalline *N*-phenyl-2,3,4,6-tetra-*O*-methyl-D-mannosylamine which upon recrystallization from acetone had m.p. 141–142°, $[\alpha]_D^{25}$ $-86 \rightarrow -8°$ in methanol (*c*, 2.5): Lit. value for 2,3,4,6-tetra-*O*-methyl-D-mannose, R_f 0.78 in solvent D[11] $[\alpha]_D$ $+11.5 \rightarrow +2.5$ in water[24]; for *N*-phenylgylcosylamine, m.p. 142–143°, $[\alpha]_D$ $-87.9 \rightarrow -8.3°$ in methanol.[25]

(b) *Component 2,* R_f 0.70–0.71 in solvent D, 494 mg. The sirup after drying had $[\alpha]_D^{25}$ +116° in water (*c*, 2.8). Demethylation of a portion (275 mg.) gave galactose and no other hexose. The galactose was separated from the other partial demethylation products and transformed in the usual manner to mucic acid, m.p. and mixed m.p. 218°. Treatment of the methylated sugar with aniline gave *N*-phenyl-2,3,4,6-tetra-*O*-methyl-D-galactosylamine, m.p. 191–192°, $[\alpha]_D^{25}$ $-80 \rightarrow +40°$ in acetone (*c*, 2.5): Lit. value for 2,3,4,6-tetra-*O*-methyl-D-galactose, R_f 0.68 in solvent D,[20] $[\alpha]_D$ $+142 \rightarrow +118°$ in water[26]; for *N*-phenylglycosylamine, m.p. 192°, $[\alpha]_D$ $-83 \rightarrow +41°$ in acetone.[25]

(c) *Component 3,* R_f 0.55–0.56 in solvent D, 21 mg. After drying the sirup had $[\alpha]_D^{25}$ +15° in water (*c*, 1.1). Demethylation of a few milligrams of the compound resulted in the formation of a component which chromatographically corresponded to mannose. Treatment of the methylated sugar with aniline resulted in the formation of some crystals which after washing with cold petroleum ether (b.p. 40–60°) and drying on a porous plate had m.p. 131–133°. The aniline condensation product had $[\alpha]_D^{25}$ +7.5 in methanol (*c*, 1.5): Lit. value for 2,4,6-tri-*O*-methyl-D-mannose, R_f 0.55 in solvent D,[13] $[\alpha]_D$ $+21 \rightarrow +14°$ in water[27]; for *N*-phenylglycosylamine, m.p. 134°, $[\alpha]_D$ $-150 \rightarrow +8°$ in methanol.[27]

(d) *Component 4,* R_f 0.49–0.50 in solvent D, 542 mg. After drying, the sirup had $[\alpha]_D^{25}$ +18.5° in water (*c*, 4). Demethylation of a portion (300 mg.) of the component gave mannose and a small amount of galactose. Other partial demethylation products were also observed on the chromatograms. The mannose was separated chromatographically and readily formed a phenylhydrazone, m.p. and mixed m.p. 198–200°. Treatment of the methylated sugar with aniline gave *N*-phenyl-2,3,6-tri-*O*-methyl-D-mannosylamine, m.p. 130–132°, $[\alpha]_D^{25}$ $-145 \rightarrow -37°$ in methanol (*c*, 1.2). The residual sirup from which no further crystalline material could be obtained had $[\alpha]_D^{25}$ −10° in methanol (*c*, 2.7). Lt. value for 2,3,6-tri-*O*-methyl-D-mannose, R_f 0.50 in solvent D,[13] $[\alpha]_D$ +6, −10° in water[28]; for *N*-phenylglycosylamine, m.p. 127–128, 133, $[\alpha]_D$ $-155 \rightarrow -39°$ in methanol.[28-30]

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(20) D. R. Briggs, E. F. Garner, R. Montgomery, and F. Smith, *Anal. Chem.*, **28**, 1333 (1956).

(21) M. J. Boyd and M. A. Logan, *J. Biol. Chem.*, **146**, 279 (1942); Marguerite Lambert and A. C. Neish, *Can. J. Res.*, **28B**, 83 (1950).

(22) W. N. Haworth, *J. Chem. Soc.*, **107**, 8 (1915).

(23) R. Kuhn, I. Low, and N. Trischmann, *Ber.*, **90**, 203 (1957).

(24) B. C. Hendricks and R. E. Rundle, *J. Am. Chem. Soc.*, **60**, 2563 (1938).

(25) J. C. Irvine and D. McNicoll, *J. Am. Chem. Soc.*, **97**, 1449 (1910).

(26) F. Smith, *J. Chem. Soc.*, 744 (1939); W. N. Haworth and Grace C. Leitch, *J. Chem. Soc.*, 188 (1918); E. L. Hirst, J. K. N. Jones, and W. O. Walder, *J. Chem. Soc.*, 1443 (1947).

(27) W. N. Haworth, R. L. Heath, and S. Peat, *J. Chem. Soc.*, 833 (1941).

(28) W. N. Haworth, E. L. Hirst, and M. M. T. Plant, *J. Chem. Soc.*, 1354 (1931); E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1278 (1948).

(29) W. N. Haworth, E. L. Hirst, and H. R. L. Streight, *J. Chem. Soc.*, 1349 (1931).

(30) F. Smith, *J. Am. Chem. Soc.*, **70**, 3249 (1948).

(e) *Component 5, R_f* 0.21–0.22, 417 mg. After drying, the sirup had $[\alpha]^{25}_{D}$ −15° in water (c, 5.2) and +5.7° in methanol (c, 5.2). Demethylation of a portion (215 mg.) of the methylated sugar afforded mannose as the only hexose and other partial demethylation products. The mannose was separated from the other components by paper chromatography and readily gave a phenylhydrazone, m.p. and mixed m.p. 198–200°, $[\alpha]^{25}_{D}$ +33° in pyridine (c, 2.0). Treatment of the methylated sugar with *p*-nitrobenzyl chloride and pyridine gave crystalline 1,4,6-tri-*p*-nitrobenzoyl-2,3,-di-*O*-methyl-D-mannose whch had m.p. 191–193°, $[\alpha]^{25}_{D}$ +64° in chloroform (c, 3.0): Lit. value for 2,3-di-*O*-methyl-D-mannose, R_f 0.22 in solvent D,[13] $[\alpha]_D$ −15.8° in water[31]; for 1,4,6-tri-*p*-nitrobenzoate, m.p. 194°, $[\alpha]_D$ +65° in chloroform.[5]

A summary of the quantities of the components obtained from the hydrolyzate of the methylated gum and the observed R_f values in solvent D is given below.

Component	R_f, Solvent D	Weight, Mg.	Mmoles
Unknown a	0.82	16	—
1	0.78–0.79	116	0.49
2	0.70–0.71	494	2.09
3	0.55–0.56	21	0.09
4[a]	0.49–0.50	542	2.46
5	0.20–0.21	417	2.00
Unknown b	0.11–0.12	14	—

[a] Contains some methylated galactose derivative, presumably 2,3,6- or 2,4,6- or both.

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(31) G. T. Robertson, *J. Chem. Soc.*, 330 (1934).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STATE UNIVERSITY COLLEGE OF FORESTRY AT SYRACUSE UNIVERSITY]

Polymerization of Anhydro Sugar Derivatives. III. 1,6-Anhydro-β-D-galactopyranose and Its 2-*O*-Methyl Ether

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The polymerization of D-galactosan to high molecular weight branched polysaccharides is described. The optical rotation of the product shows the presence of a mixture of α and β linkages. Periodate oxidation indicates a product with forty-three of one hundred units unsubstituted on the secondary hydroxyls, fifty-six substituted on C-2 or C-4 and only one unit resistant to periodate (substituted on C-3 or disubstituted). The 2-*O*-methyl ether is very resistant to polymerization presumably because transformation of the 1,6-anhydro ring to the 1,2-anhydro ring is impossible.

In a reinvestigation[2] of Pictet's[3] polymerization of levoglucosan, it has been shown that highly branched glucosans are produced linked primarily 1,6- and to a lesser degree in the 2,4- and probably 3-positions. Both α and β anomeric forms were shown to be present and these conclusions were generally confirmed and extended by Wolfrom and co-workers in a concurrent research.[4,5] The highest weight average molecular weights achieved in this laboratory were about 20,000 as measured by means of the ultracentrifuge. The products were contaminated by a small percentage of microgel byproduct which was eliminated in the ultracentrifuge determination but which caused molecular weights measured by light scattering to be substantially higher.[6]

In order to explain the presence of α linkages in the polymer, we have proposed a reaction mechanism involving the interaction of the 2-hydroxyl group with the C-1 carbon atom in the transition state. Abe and Prins point out that the molecular weight distribution is most readily explained if, concurrent to our proposed reaction, a more rapid dimerization occurs by the reaction of two anhydro rings. The reactive intermediate is assumed then to attack hydroxyl groups indiscriminately.

In order to gain further perspective on the mechanism of this reaction and to obtain further examples, we now have studied the polymerization of 1,6-anhydro-β-D-galactose and its 2-*O*-methyl ether.

EXPERIMENTAL

Preparation and isolation of monomers and polymers. D-Galactosan and its 2-*O*-methyl ether were prepared respectively by pyrolysis of α-lactose monohydrate[7] and by dimethyl sulfate methylation and hydrolysis of 3,4-isopropylidene-D-galactosan.[8] The physical properties of the former were: m.p. 223–224° $[\alpha]^{24}_{D}$ −22° (c = 1.96) and the latter:

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(2) J. daS. Carvalho, W. Prins, and C. Schuerch, *J. Am. Chem. Soc.*, **81**, 4054 (1959).

(3) (a) A. Pictet and J. Sarasin, *Helv. Chim. Acta*, **1**, 87 (1918); (b) A. Pictet, *Helv. Chim. Acta*, **1**, 226 (1918); (c) A. Pictet and J. Pictet, *Helv. Chim. Acta*, **4**, 788 (1921); (d) A. Pictet and J. Pictet, *Compt. rend.*, **173**, 158 (1921); (e) A. Pictet and J. H. Ross, *Compt. rend.*, **174**, 1113 (1922); (f) A. Pictet and J. H. Ross, *Helv. Chim. Acta*, **5**, 876 (1922); (g) Hoffman-LaRoche and Co., A.G., German Pat. **513,126** (Feb. 29, 1928).

(4) A. Thompson and M. L. Wolfrom, *J. Am. Chem. Soc.*, **80**, 6618 (1958).

(5) M. L. Wolfrom, A. Thompson, and R. B. Ward, *J. Am. Chem. Soc.*, **81**, 4623 (1959).

(6) H. Abe and W. Prins, *Makromol. Chem.*, XLII, 216 (1961).

(7) R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 2435 (1942).

(8) R. E. Reeves, *J. Am. Chem. Soc.*, **71**, 2118 (1949).