377. *Cholla Gum.*

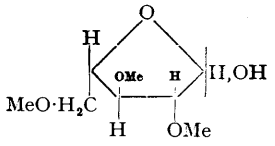
By F. Brown, E. L. Hirst, and J. K. N. Jones.

Cholla gum, an exudate of the cactus, *Opuntia fulgida*, has been shown to consist of L-arabinose (6 parts), D-xylose (2 parts), L-rhamnose (trace), D-galactose (3 parts), and D-galacturonic acid (1 part) in the approximate proportions indicated. Hydrolysis of the methylated derivative of cholla gum yields 2 : 3 : 5-trimethyl L-arabinose (4 parts), 2 : 3 : 4-trimethyl D-xylose (2 parts), 2 : 3-dimethyl L-arabinose (1 part), 2 : 4-dimethyl D-galactose (3 parts), L-arabinose (1 part) and 2-methyl D-galacturonic acid (1 part). The L-arabinose residues are present in the furanose form, and the remainder of the sugars are in the pyranose form. Cholla gum, therefore, resembles other plant gums in many features of its architecture, the main difference being that its acidity is due to D-galacturonic acid instead of D-glucuronic acid.

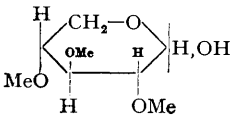
The work of Smith (*J.*, 1939, 744, 1724; 1940, 1035) and Jackson and Smith (*J.*, 1940, 74, 79) on the structure of gum arabic, an exudate of a tropical member of the family Leguminosæ has shown that the polysaccharide resembles closely the gums exuded by trees of the family Rosaceæ growing in temperate climates (Hirst, *J.*, 1942, 70). Cholla gum is of tropical origin and is exuded on the white cactus, *Opuntia fulgida*, a native of Mexico and of the Southern States of America, and it was of interest, therefore, to see whether a similar type of structure is present. The formation of the gum seems to be favoured by prolonged hot dry spells and, although its function in the economy of the cactus is unknown, it may be that in periods of drought it enables the plant to resist desiccation. Through the kindness of Dr. K. Folkers, and of Dr. B. A. Krukoff of the New York Botanical Garden, we were able to obtain samples of this material. Cholla gum has been examined previously by Anderson and Sands (*Amer. J. Pharm.*, 1925, **97**, 589) and by Klaas and Sands (*J. Amer. Chem. Soc.*, 1929, **51**, 3441), but as the properties of their material differed in some respects (particularly in optical rotation) from our sample we decided to examine our polysaccharide in some detail. The gum was purified by precipitation from acid

solution by the addition of alcohol, yielding an acidic polysaccharide as a white powder which was easily soluble in water and showed a negative rotation ($[\alpha]_D$ $-80°$). Similar analyses were obtained on examination of samples prepared from different nodules of the crude gum. One component only was detected on electrophoretic examination, carried out by Dr. F. A. Isherwood, to whom we are indebted for these results. On hydrolysis with dilute acid the gum gave a mixture of sugars amongst which L-arabinose, D-xylose, D-galactose, and D-galacturonic acid were detected. A trace of L-rhamnose was also present. It may be noted that Klaas and Sands (*loc. cit.*) did not detect D-xylose but found instead L-rhamnose (5·5%) in the products of hydrolysis of the sample they examined. The separation, identification, and estimation of the component sugars of the polysaccharide was greatly facilitated by the use of the paper chromatographic technique (Partridge, *Nature*, 1946, **158**, 270; *Biochem. J.*, 1948, **42**, 238; Flood, Hirst, and Jones, *Nature*, 1947, **160**, 86; *J.*, 1948, 1679). By this method it was shown that our sample of cholla gum contained the following sugars in the proportions indicated: L-arabinose (6 parts), D-xylose (2 parts), D-galactose (3 parts), D-galacturonic acid (1 part), and L-rhamnose (trace). Traces of other sugars may also be present. On boiling the gum acid with dilute acids, L-arabinose, D-xylose, and D-galactose were first liberated leaving a more resistant nucleus of D-galactose and D-galacturonic acid which was split on further hydrolysis with stronger acid into its component parts. It follows that Cholla gum resembles closely other plant gums (cf. Hirst and Jones, *J. Soc. Dyers and Col.*, 1947, **63**, 249) in that it contains D-xylose and L-arabinose, both of which are liberated from the polysaccharide on acidic hydrolysis with relative ease. It differs, however, from other gums examined by us in the structure of the nucleus, which is more resistant to acidic hydrolysis and consists of residues of D-galactose and D-galacturonic acid. D-Glucuronic acid is absent, cholla gum displaying in this respect a similarity to the plant mucilages rather than to the gums hitherto examined (compare, however, gum tragacanth, which also contains D-galacturonic acid; James and Smith, *J.*, 1945, 739).

Information concerning the mode of linkage of the various component sugars in the molecule was obtained from an examination of the products of hydrolysis of the fully methylated polysaccharide. As was expected, hydrolysis of the methylated derivative gave a complex mixture of sugar derivatives, which could be separated in the pure state only with great difficulty. Quantitative estimates of the proportions in which the various sugar residues are present can, therefore, be only approximate at this stage. Nevertheless, from a study of the physical constants of the various sugars identified and from an examination of the different fractions obtained by extraction and distillation of the mixture produced on methanolysis of the polysaccharide a rough estimate can be made. By these methods of fractionation, followed by hydrolysis of the methylglycosides, the following sugars were identified in the approximate molecular proportions given in parentheses: (1) 2 : 3 : 5-trimethyl L-arabinose (I) (4 parts), identified, after oxidation with bromine water, as the crystalline lactone of 2 : 3 : 5-trimethyl L-arabonic acid; (2) 2 : 3 : 4-trimethyl D-xylose (II) (2 parts), isolated as the crystalline sugar, whose identity was confirmed by oxidation to the crystalline lactone of 2 : 3 : 4-trimethyl D-xylonic acid; (3) 2 : 3-dimethyl L-arabinose (1 part) [shown in (III) as the furanose form to indicate the mode of linkage in the polysaccharide], identified after oxidation to 2 : 3-dimethyl L-arabonic acid and conversion into the amide; (4) 2 : 4-dimethyl D-galactose (IV) (3 parts), whose identity was



(I.)



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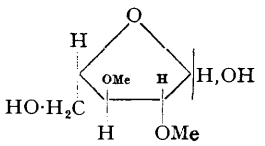

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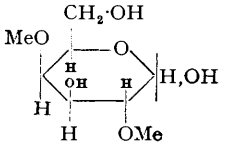

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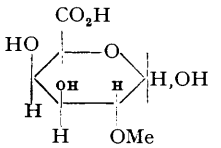

(V.)

confirmed by isolation of the crystalline sugar, by conversion into the crystalline anilide, and by oxidation of the sugar to 2 : 4-dimethyl D-galactonic acid, identified as its crystalline amide;

(5) L-arabinose (1 part) (unsubstituted), isolated as its benzoylhydrazone; and (6) 2-methyl D-galacturonic acid (V) (1 part), identified as the diamide of 2-methyl D-galactosaccharic acid. 2 : 4-Dimethyl D-galactose was also found combined with 2-methyl D-galacturonic acid in the form of an aldobionic acid [compare the aldobionic acid derivative produced on hydrolysis of degraded egg plum gum (Hirst and Jones, *J.*, 1948, 120)].

The D-xylose residues are present in the pyranose form as end-groups, and the major portion (probably all) of the L-arabinose residues are of the furanose type and most of them are present as end-groups. A proportion of the arabinose is combined in the furanose form through $C_{(1)}$, $C_{(2)}$, $C_{(3)}$, and $C_{(5)}$ (V). This type of combination in which every hydroxyl group, other than that concerned with ring formation, is combined with another sugar residue has hitherto been encountered only rarely, but it is found in the polysaccharide present in ovomucoid (Stacey and Wooley, *J.*, 1942, 550) and in slippery-elm mucilage (Brown, Hirst, Hough, Jones, and Wadman, *Nature*, 1948, **161**, 720).

In the present instance the absence of monomethyl arabinose is further evidence that fully substituted arabinose residues are actually components of the gum molecule and that the isolation of the free arabinose after hydrolysis is not to be attributed to incomplete methylation.

Cholla gum resembles damson gum (Hirst and Jones, *J.*, 1946, 506) in that it contains L-arabofuranose residues linked through $C_{(5)}$ and $C_{(1)}$ (III). The D-galactose residues are triply linked through $C_{(1)}$, $C_{(3)}$, and $C_{(6)}$ (IV), $C_{(2)}$ and $C_{(4)}$ of the D-galactose being unsubstituted in the gum molecule. It appears, therefore, that D-galactose favours the 1 : 3- and 1 : 6-type of linkage typical of its occurrences in other gums. The uronic acid residue (V) is linked through three positions $C_{(1)}$, $C_{(3)}$, and $C_{(4)}$. On only one other occasion hitherto has this acid been detected amongst the products of hydrolysis of methylated gum, namely by James and Smith (*loc. cit.*) in the hydrolysis products of methylated tragacanthic acid. Cholla gum, therefore, differs in this respect from the other gums which have been investigated, since these have contained D-glucuronic acid residues linked only through either $C_{(1)}$ or $C_{(1)}$ and $C_{(4)}$. Cholla gum, therefore, shows certain divergences from the gums exuded on trees of the family Rosaceæ in temperate climates.

At this stage it is not possible to formulate a unique structure for the polysaccharide, but the isolation of large amounts of end-group (I and II) shows that the molecule must be of the highly branched type.

Experimental.

Purification of the Gum.—The crude gum was dark brown, and has a characteristic odour. It was purified by dissolving the powdered material in warm dilute aqueous sodium hydroxide, during which process ammonia was evolved and silica was precipitated. It is not known whether this silica was combined with the gum or whether it was dust which had been trapped in the sticky gum when it was first exuded. The solution of gum was then filtered, and the cooled filtrate acidified with cold dilute hydrochloric acid, whereupon the colour changed from dark brown to clear light yellow. The polysaccharide was precipitated by the addition of methyl alcohol (3 volumes) to the vigorously stirred solution. The product was washed twice by decantation, filtered off, dissolved in cold water, and reprecipitated by the addition of methyl alcohol with stirring. The pale cream-coloured product was filtered off, washed with acetone and ether, and dried at 40° in a vacuum-oven. Four separate nodules in addition to a large batch of gum were purified in this manner. No significant differences in properties were observed. The pure gum had $[\alpha]_D^{15°}$ −82° in water (as sodium salt), $[\alpha]_D^{15°}$ −83° in water as free acid; furfuraldehyde liberated by boiling 12% hydrochloric acid, 40%; uronic anhydride, 10·4%; OMe, <1%; N, <0·2%; ash (weighed as sulphate), 2·5%.

Examination of a sample of the gum in the Tiselius electrophoretic apparatus indicated that the polysaccharide was homogeneous (experiments carried out by Dr. F. A. Isherwood).

Hydrolysis of the Gum.—The purified gum (3·65 g.) was dissolved in 0·01N-sulphuric acid (100 c.c.), and the solution was boiled under reflux. The viscosity of the solution fell rapidly. $[\alpha]_D^{11°}$ were −82° (initial value), −66° (1 hour), −23° (2½ hours), +4° (4½ hours), +25° (6¼ hours), +37° (7½ hours), +49° (8½ hours), +51° (9½ hours), +53° (10 hours). Hydrolysis was much slower after this stage. The solution was neutralised with barium carbonate and filtered. The filtrate was concentrated at 40°, under reduced pressure, to a syrup (4·01 g.) which was exhaustively extracted with boiling methyl alcohol, to give a soluble fraction *A* (2·71 g.), $[\alpha]_D$ +45° in water, and an insoluble fraction *B* (1·30 g.) (Found for *B*: Ba 12·3%; a trisaccharide containing two hexose units and one hexuronic acid residue requires Ba, 11·7%).

Examination of fraction A. Fraction *A* (2·71 g.) was boiled with N-sulphuric (50 c.c.), the hydrolysis being followed polarimetrically: $[\alpha]_D$ +45° (initial value); +63° (30 minutes); +67° (90 minutes) (constant value). This change in optical rotation indicates that fraction (*A*) contained some oligosaccharide. The solution was neutralised with barium carbonate and filtered, and the filtrate was evaporated to a syrup (2·71 g.; $[\alpha]_D$ +72° in water) under reduced pressure. A portion of this material was examined on the paper chromatogram which revealed the presence of arabinose, xylose, and galactose. The presence of these sugars was confirmed in the following ways: (*a*) by the isolation of crystalline L-arabinose, m. p. 154°, $[\alpha]_D^{27°}$ +103° (equilibrium value in water), and by the formation of L-arabinose benzoylhydrazone, m. p. 186° (decomp.) (0·23 g.), from a portion of the syrup (0·24 g.) (the fraction contained, therefore, 65% of L-arabinose); (*b*) D-galactose was detected and estimated as its phenylmethylhydrazone, m. p.

187° (0·53 g. syrup gave 0·63 g. of derivative corresponding to the presence of 0·049 g. of D-galactose after correcting for the presence of L-arabinose phenylmethylhydrazone; cf. Hirst, Jones, and Woods, *J.*, 1947, 1048); and (*c*) D-xylose was detected by the formation of its sparingly soluble dibenzylidene dimethyl acetal, m. p. 211°, $[\alpha]_D^{20°}$ −9° (in chloroform) (Breddy and Jones, *J.*, 1945, 738; Wise and Ratcliff, *Ind. Eng. Chem., Anal. Ed.*, 1947, **19**, 694). An independent estimation of the sugars was carried out by the paper chromatogram method (Flood, Hirst, and Jones, *loc. cit.*). The sugars were found to be present in the relative proportions, L-arabinose (6 parts), D-xylose (2 parts), and D-galactose (1 part).

Examination of insoluble fraction B. A portion (0·52 g.) of this fraction was dissolved in 0·1N-sulphuric acid, and the precipitated barium sulphate filtered off. The filtrate was boiled under reflux, and the reaction was followed polarimetrically: $[\alpha]_D$ +38° (initial value); +49° (2 hours); +61° (5 hours); +74° (9 hours); +76° (10¾ hours). The cooled solution was neutralised with barium carbonate and filtered, and the filtrate concentrated under reduced pressure. The resulting syrup (0·53 g.) was extracted with boiling methyl alcohol, and the extracts were concentrated under reduced pressure to a syrup (0·17 g.), $[\alpha]_D$ +75° (in water), which, by examination on the paper chromatogram, was found to contain mainly D-galactose, with traces of arabinose, xylose, and rhamnose. The undissolved barium salt [0·26 g.; $[\alpha]_D$ +73° (in water) (Found: Ba, 17·3%; the barium salt of an aldobionic acid requires Ba, 16·2%)] was hydrolysed by boiling 2N-sulphuric acid (6·3 c.c.), the hydrolysis being followed polarimetrically: $[\alpha]_D$ +74° (initial value); +68° (30 minutes); +53° (1 hour); +52° (1¾ hours, constant value). The cooled solution was neutralised with barium carbonate and filtered, and the filtrate evaporated giving a solid (0·11 g. from 0·18 g. of barium salt) which was extracted with methyl alcohol. The extracted material (0·044 g.) was a syrup which contained galactose and a trace of rhamnose, both identified on the paper chromatogram. The residual barium salt (0·068 g.), $[\alpha]_D$ +74° (in water) (Found: Ba, 27·1; the barium salt of a hexuronic acid requires Ba, 26·2%), was converted into the acid by adding the calculated quantity of 0·01N-sulphuric acid and the filtered solution was treated with bromine water. After several days the precipitate of mucic acid was filtered off, washed, and dried; m. p. 215°, undepressed on admixture with an authentic specimen. The presence of barium D-galacturonate was confirmed by the formation of a brick-red precipitate on warming the barium salt with basic lead acetate solution (Ehrlich, *Ber.*, 1932, **65**, 352).

Methylation of Cholla Gum.—The purified gum (25 g.) was dissolved in water (300 c.c.) and methylated at 20° by the portionwise addition, with vigorous stirring, of 30% aqueous sodium hydroxide (400 c.c.) and methyl sulphate (200 c.c.). After addition of the reagents stirring was continued for 12 hours and the solution was then concentrated on a steam-bath. The concentrate was treated (conditions as above) with methyl sulphate (200 c.c.) and 30% aqueous sodium hydroxide (400 c.c.). After two further methylations a crude gummy product was isolated by heating the solution on the boiling water-bath. Methylation was completed by evaporating to dryness a solution of the material in benzene containing thallous ethoxide (1·7N.; 100 c.c.), and boiling the residual solid (powdered, 80 mesh) with methyl iodide, until the insoluble material no longer gave an alkaline reaction to litmus paper. The methylated derivative was isolated by concentration of the filtered solution (Found: OMe, 38·4%). An attempt was made to fractionate the methylated polysaccharide by heating it with N-sodium hydroxide (50 c.c.) and extracting the resultant solution with chloroform. Concentration of the extract gave a fraction of methylated cholla gum (I) which had properties very similar to those of the fraction II obtained after further extraction of the acidified aqueous solution [fraction I, $[\alpha]_D^{20°}$ − 110° ± 5° (in methyl alcohol) (Found: OMe, 38·4%); fraction II, $[\alpha]_D^{20°}$ − 100° ± 5° (in methyl alcohol) (Found: OMe, 38·1%)]. In view of the similarity in properties and of the failure to detect heterogeneity by electrophoretic experiments on the original gum, these fractions were recombined for further examination.

Hydrolysis of the Methylated Polysaccharide.—The following details are typical of several experiments. Methylated cholla gum (14·17 g.) was dissolved in methanolic hydrogen chloride (1%; 300 c.c.), and the solution was boiled under reflux for 8 hours. It was not possible to follow the reaction polarimetrically owing to the darkness of the solution. The solution was then cooled and hydrogen chloride was removed by the addition of an ice-cold solution of diazomethane in ether. The solvents were removed by evaporation on the boiling water-bath at atmospheric pressure. The last traces of solvent were removed under reduced pressure, and the residual syrup was heated with 0·3N-barium hydroxide (60 c.c.) at 60° for 3 hours to convert the esters of the uronic acid derivatives into the corresponding barium salts. A stream of carbon dioxide was then passed through the solution to remove the excess of barium hydroxide, and the solution was filtered. The filtrate was exhaustively extracted with purified light petroleum (b. p. 40—60°), yielding a fraction (*A*) (7·15 g.), $n_D^{20°}$ 1·4353, soluble in light petroleum. Extraction of the aqueous solution with ether then yielded an ether-soluble fraction (*B*) (1·38 g.), $n_D^{20°}$ 1·4490. The aqueous solution was then evaporated to dryness, and the solid residue was exhaustively extracted with dry ether, yielding a soluble fraction (*C*) (3·89 g.) and an insoluble residue (*D*) (2·9 g.), which was purified by dissolving it in methyl alcohol and filtering off traces of barium carbonate. The product, after removal of the methyl alcohol, had Ba, 17·3, and OMe, 22·3%.

Examination of fractions A *and* B. (All distillation temperatures recorded are bath temperatures.) Fraction *A* (7·15 g.) was distilled from a Widmer flask, yielding fraction I (6·65 g.), b. p. 150°/20 mm., $n_D^{20°}$ 1·4347, $[\alpha]_D^{20°}$ −43° (in water) (Found: OMe, 60·0%). Fraction *B* was then added to the Widmer flask, and the distillation was continued yielding fraction II*a* (0·46 g.), b. p. 150—160°/24 mm., $n_D^{21°}$ 1·4362 (Found: OMe, 57·0%). A portion (1·06 g.) of the still-residue (1·43 g.) was further fractionated by dissolving it in water and extracting the solution with light petroleum (b. p. 40—60°) for several hours (continuous extraction apparatus). Evaporation of the solvent left a fraction II*b* (0·29 g.) (Found: OMe, 59·8%). The aqueous solution on evaporation gave a syrup (0·75 g.) which was purified by distillation giving fraction III, b. p. 120°/0·1 mm., $n_D^{19°}$ 1·4490 (Found: OMe, 45·2%).

Examination of the fractions produced on distillation. (a) *Fraction* I. A portion (0·13 g.) was boiled with N-sulphuric acid (10 c.c.), the reaction being followed polarimetrically: $[\alpha]_D$ −43° (initial value); −19° (½ hour); −20° (1 hour); −21° (2 hours; constant value). The cooled solution was neutralised with barium carbonate and filtered, and the filtrate evaporated to dryness under reduced pressure. The

residue was extracted with acetone, and the sugars which dissolved were then purified by distillation; $n_D^{19°}$ 1·4522, $[\alpha]_D$ −22° (*c*, 0·6 in water) (Found: OMe, 47·8. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). These figures indicated that the fraction was a mixture of 2 : 3 : 5-trimethyl L-arabinose and 2 : 3 : 4-trimethyl D-xylose. Accordingly a second portion (5·30 g.) of fraction I was boiled in 0·4N-hydrobromic acid (50 c.c.) for 8 hours. Trial experiments had shown that under these conditions methylarabofuranoside was completely hydrolysed, whilst methylxylopyranoside was only partly hydrolysed. $[\alpha]_D$ were −43° (initial value), −39° (1 hour), −25° (3 hours), −18° (5½ hours), −17° (7 hours), and −17° (8 hours). The cooled solution was neutralised with N-sodium hydroxide and evaporated to a syrup under reduced pressure, and the sugars were extracted with acetone and distilled in a vacuum, yielding fraction A_1 (0·61 g.), b. p. 160°/12 mm., $n_D^{16°}$ 1·4490, fraction A_2 (2·34 g.), b. p. 130°/0·2 mm., $n_D^{16°}$ 1·4515, and a still-residue (1·81 g.). The refractive index of fraction A_1 suggested that it contained an appreciable proportion of 2 : 3 : 5-trimethyl L-arabinose and 2 : 3 : 4-trimethyl D-xylose, admixed with the fully methylated methylxyloside. This fraction (A_1) was, therefore, dissolved in water and extracted with light petroleum (b. p. 40—60°) for 2 hours. The extracts on concentration gave a syrup (0·27 g.), $n_D^{18°}$ 1·4440, which was hydrolysed with boiling N-sulphuric acid for 3 hours. Removal of the sulphuric acid as barium sulphate, followed by evaporation of the aqueous solution, gave 2 : 3 : 4-trimethyl D-xylose, m. p. 91° (after recrystallisation from ether), not depressed on admixture with an authentic specimen. Oxidation of this material with bromine water gave 2 : 3 : 4-trimethyl D-xylonolactone, m. p. 55°, not depressed on admixture with an authentic specimen.

Fraction A_2 and the still-residue were combined and oxidised with bromine water until the solution was non-reducing. Bromine was removed by aëration, and the solution was neutralised with silver carbonate and filtered. Hydrogen sulphide was passed through the solution, and the precipitated silver sulphide was filtered off. The filtrate on evaporation gave crystalline 2 : 3 : 5-trimethyl L-arabonolactone, m. p. and mixed m. p. 31°. This was converted by methanolic ammonia into the crystalline amide, m. p. and mixed m. p. 138° (Found: N, 6·4; OMe, 45·3. Calc. for $C_8H_{17}O_5N$: N, 6·7; OMe, 44·5%).

From the equilibrium rotation of the product obtained by acidic hydrolysis of fraction I ($[\alpha]_D$ −22°), it was calculated that this fraction contained 2 : 3 : 5-trimethyl L-arabinose and 2 : 3 : 4-trimethyl D-xylose in the approximate ratio of 2 : 1.

Fractions IIa *and* IIb. These fractions were combined, and a portion (0·29 g.) was boiled with N-sulphuric acid (10 c.c.) for 3 hours; $[\alpha]_D^{20°}$ −30° (initial value), −12° (end value). The cooled solution was neutralised with barium carbonate and filtered and the filtrate was concentrated to a syrup, $[\alpha]_D$ −13° (in water). When this was set aside, crystalline 2 : 3 : 4-trimethyl D-xylose separated and was isolated on porous tile. After recrystallisation from ether it had m. p. 91—92°, not depressed on admixture with an authentic specimen. The non-crystalline material was oxidised with bromine water, and the crude lactone was purified by distillation. The distillate crystallised when kept, giving 2 : 3 : 5-trimethyl L-arabonolactone, m. p. 29°, which was converted by liquid ammonia into 2 : 3 : 5-trimethyl L-arabonamide, m. p. 137°, not depressed on admixture with an authentic specimen.

Fraction III. A portion (0·13 g.) of this fraction was hydrolysed with N-sulphuric acid, the reaction being followed polarimetrically: $[\alpha]_D$ −12° (initial value); +75° (1 hour); +76° (1½ hours, constant value). The solution was neutralised with barium carbonate. Concentration after filtration gave a syrupy mixture of reducing sugars, $n_D^{19°}$ 1·4738 (Found: OMe, 33·7. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). When a portion of this syrup (0·10 g.) was boiled with alcoholic aniline (0·05 g. in 5 c.c.) the anilide of 2 : 3-dimethyl L-arabinose was formed, and crystallised on removal of the solvent; m. p. 137° after recrystallisation from ethyl alcohol, not depressed on admixture with an authentic specimen. A further portion of the syrup (0·16 g.) was oxidised with bromine water, and the lactone of the resultant acid was isolated as described above. On treatment with liquid ammonia the lactone gave 2 : 3-dimethyl L-arabonamide, m. p. (after recrystallisation from acetone–ether) 156°, not depressed on admixture with an authentic sample. It was calculated that fraction III contained about 90% of 2 : 3-dimethyl L-arabinose.

Examination of fraction C. This fraction (3·89 g.) was partly crystalline. The crystalline component (0·22 g.) was separated on porous tile and identified as 2 : 4-dimethyl β-methyl-D-galactoside, m. p. and mixed m. p. 162—164°, $[\alpha]_D$ −4° (in water) (Found: OMe, 42·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). This was confirmed by hydrolysing a portion (0·13 g.) with boiling N-sulphuric acid (5 c.c.) for 1 hour: $[\alpha]_D$ −4° ⟶ +77°. Removal of the sulphuric acid as barium sulphate, followed by concentration of the aqueous solution, gave crystalline 2 : 4-dimethyl D-galactose monohydrate, m. p. 104°. The non-crystalline material (3·64 g.) was isolated by exhaustive extraction of the tile with chloroform. The methoxyl content (36·3%) of this syrup showed that some material of lower methoxyl content than dimethyl methyl-D-galactoside was present. This was identified as methyl-L-arabinoside in the following way. A portion of the syrup (1·21 g.) was boiled with N-sulphuric acid ($[\alpha]_D^{20°}$ changed from + 76° to +80°, constant value, in 1½ hours). The syrupy product, isolated in the usual manner, had $[\alpha]_D$ +86° (in water) and the methoxyl content (25·8%) again indicated the presence of some other sugar of a lower methoxyl content than dimethyl D-galactose (OMe, 29·8%). A portion of the syrup crystallised when kept for several days, and the crystals were separated on porous tile. Recrystallisation from acetone gave pure 2 : 4-dimethyl D-galactose monohydrate, m. p. and mixed m. p. 104° [Anilide, m. p. 205° (Found: N, 5·2; OMe. 22·1. Calc. for $C_{14}H_{21}O_5N$: N, 5·0; OMe, 21·9%)]. The tile was extracted with methanol, and the extract boiled with methanolic hydrogen chloride (3%). After four hours the solution was cooled, neutralised with silver carbonate, and filtered. On evaporation of the solvent a syrup was obtained from which crystalline β-methyl-L-arabinoside separated, m. p. and mixed m. p., after recrystallisation from aqueous alcohol, 168—170° (Found: OMe, 18·7. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). It is evident, therefore, that the presence of methyl-L-arabinoside in the fraction C is, in part at least, responsible for the low methoxyl content. An estimate of the amount of 2 : 4-dimethyl methyl-D-galactoside present in the syrup removed from the tile may be made from its methoxyl content (36·3%) and from the methoxyl content (25·8%) of the sugars produced on hydrolysis. These figures indicate the presence of *ca.* 85% of the galactose derivative and 15% of the arabinose derivative. This conclusion was confirmed by oxidising a portion (0·313 g.) of the reducing sugars with 0·3M-sodium

periodate (10 c.c.) and determining the formic acid produced [Found: 5·86 mg. ($\frac{1}{2}$ hour); 6·38 mg. (1 hour); 7·89 mg. (24 hours)]. A blank experiment on L-arabinose (28 mg.) under similar conditions showed that 74% of the calculated quantity of formic acid is produced in 24 hours. Approximately 14% of L-arabinose was present, therefore, in the mixture of reducing sugars.

Examination of residue D. The analytical figures (Found: Ba, 17·3; OMe, 22·3%) indicated that this fraction was not the pure barium salt of a methylated hexuronic acid (Ba content too low). A portion (1·31 g.) was exhaustively extracted with ether in a Soxhlet apparatus. Evaporation of the ether left a syrup (0·16 g.) identified as 2 : 4-dimethyl methyl-D-galactoside, since on hydrolysis with N-sulphuric acid it gave crystalline 2 : 4-dimethyl D-galactose monohydrate, m. p. and mixed m. p. 104°. The residual barium salt (Found: Ba, 19·0%) was boiled with 4% methanolic hydrogen chloride (25 c.c.) for 8 hours to convert the uronic acid residue into the corresponding methyl ester and to hydrolyse any oligosaccharide. The cooled solution was neutralised with silver carbonate and filtered, and the filtrate evaporated to dryness and dissolved in warm 0·3N-barium hydroxide (30 c.c.) in order to convert any ester into the barium salts. Excess of barium hydroxide was removed with carbon dioxide, and the filtered solution was evaporated to dryness. The residue was extracted exhaustively with ether (continuous extraction apparatus) to remove 2 : 4-dimethyl methyl-D-galactoside (0·22 g.) produced by methanolysis. The residual barium salt (0·91 g.) now analysed as the barium salt of the methylglycoside of a monomethyl galacturonic acid (Found: Ba, 24·6; OMe, 21·3. Calc. for $C_8H_{13}O_7Ba_{\frac{1}{2}}$: Ba, 23·7; OMe, 21·7%). This salt (0·90 g.) was converted into the corresponding ester by boiling methanolic hydrogen chloride (1%; 3 hours). Barium chloride was removed by filtration and the solution was neutralised with silver carbonate and filtered. Evaporation of the solvent now gave a syrup which was distilled in a micro-distillation apparatus (Brown and Jones, *loc. cit.*); b. p. 180°/1 mm., $n_D^{20°}$ 1·4695 (Found: OMe, 38·5. Calc. for $C_9H_{16}O_7$: OMe, 39·4%). The ester on reaction with liquid ammonia gave an amide, m. p. 173°, in small yield. The ester (0·21 g.) was hydrolysed with N-hydrochloric acid (10 c.c.) at 100°: $[\alpha]_D^{20°}$ +21° (initial value); +32° ($1\frac{1}{4}$ hours); +33° ($3\frac{1}{4}$ hours, constant value). The cooled solution was neutralised with silver carbonate, and the solution was filtered before and after the passage of hydrogen sulphide. The filtrate was aërated to remove hydrogen sulphide, and the uronic acid was oxidised with bromine water until it no longer reduced Fehling's solution. Silver oxide was then added to remove bromide ions and to convert the acid into its silver salt. The solution was filtered before and after the passage of hydrogen sulphide, and the filtrate concentrated to a syrup which was esterified by boiling it with methyl alcoholic hydrogen chloride. The resultant ester gave, with methyl alcoholic ammonia, the diamide of 2-methyl D-galactosaccharic acid, m. p. and mixed m. p. 195° (Jones and Stacey, *J.*, 1947, 1340).

Summary of Yields.—From optical rotations and methoxyl contents of the various fractions it was calculated that the sample of methylated cholla gum (14·17 g.) had yielded, on methanolysis, approximately the following weights and molecular proportions of sugar derivatives: 2 : 3 : 5-trimethyl methyl-L-arabonoside (5·00 g., 4 mols.); 2 : 3 : 4-trimethyl methyl-D-xyloside (2·50 g., 2 mols.); 2 : 3-dimethyl methyl-L-arabinoside (1·04 g., 1 mol.); 2 : 4-dimethyl methyl-D-galactoside (4·13 g., 3 mols.); methyl-L-arabinoside (0·64 g., 1 mol.); and 2-methyl methyl-D-galacturonoside methyl ester (1·65 g., 1 mol.). These yields and molecular ratios are to be regarded as approximate, in view of the difficulty encountered in separating the various fractions.

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