408. *The Polysaccharides of Carragheen. Part III. Confirmation of the 1 : 3-Linkage in Carragheenin, and the Isolation of* L-*Galactose Derivatives from a Resistant Fragment.*

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Carragheenin, after partial hydrolysis with oxalic acid, yielded a dialysable degraded polysaccharide from which 25% of the original sulphuric ester groups had been removed. 2 : 4 : 6-Trimethyl galactose, accompanied by tetramethyl galactopyranose and 2 : 6-dimethyl galactose, was produced on hydrolysing the methylated degradation product, thus supporting the previous conclusion that the principal linkage is through $C_{(1)}$ and $C_{(3)}$ with the sulphate residue on $C_{(4)}$.

On treatment of carragheenin with methanolic hydrogen chloride at room temperature a residue (15%) with a low sulphate content (1·5%) remained, whilst methylgalactosides and sodium methyl sulphate, etc., dissolved. The resistant fragment ($[\alpha]_D$ −5°) gave an acetate which on deacetylation and methylation gave a methyl derivative, among the products of hydrolysis of which were identified 2 : 3 : 4 : 6-tetramethyl and 2 : 4 : 6-trimethyl L-galactose, as well as smaller quantities of the corresponding D-galactose derivatives, 2 : 4-dimethyl D-galactose and trimethyl D-xylopyranose. Some implications of these results are discussed.

In Parts I and II of this series (*J.*, 1943, 51; 1947, 1622) the isolation of 2 : 6-dimethyl D-galactose from the methylated polysaccharides prepared from *Chondrus crispus* and *Gigartina stellata* extracts, respectively, together with the stability to alkaline hydrolysis of the sulphate residues, provided evidence that the principal linkage between D-galactose residues in carragheenin was through $C_{(1)}$ and $C_{(3)}$ with the hydroxyl group on $C_{(4)}$ esterified by sulphate. Further support for this view has now been obtained in the isolation of 2 : 4 : 6-trimethyl galactose from specimens of carragheenin which had been partly degraded by treatment with dilute oxalic acid and then methylated and hydrolysed. The degraded carragheenin which was soluble in 80% ethanol–water and was of a sufficiently small particle size to pass through a cellophane membrane by dialysis, had lost 25% of its original bound sulphate. After methylation, hydrolysis, and glycoside formation followed by continuous extraction with suitable solvents, mixtures of methylglycosides of tetramethyl D-galactopyranose (*ca.* 12%) and 2 : 4 : 6-trimethyl D-galactose (*ca.* 18%) were separated from 2 : 6-dimethyl methylgalactosides which remained in the aqueous solution. Although the free sugars isolated on hydrolysis were homogeneous when examined on the paper chromatogram and readily gave crystalline aniline derivatives several recrystallisations were necessary before pure crystalline products were obtained. In the light of the results which follow, it seems highly probable that there was some contamination by the corresponding enantiomorphs. The main conclusion to be drawn from these experiments, however, is that the 2 : 4 : 6-trimethyl galactose is derived from units linked through $C_{(3)}$, and that these residues formerly carried a sulphate group on $C_{(4)}$, since the main product of the hydrolysis of methylated carragheenin is 2 : 6-dimethyl galactose.

Carragheenin cannot be represented simply by a chain of galactose units linked through the 1 : 3-positions. When the polysaccharide is shaken with 1% methanolic hydrogen chloride at room temperature the greater part dissolves with the production of methylgalactosides and salts of methylsulphuric acid. An insoluble residue (*ca.* 15%) remains, however, which is practically devoid of sulphate and, in contradistinction to the original carragheenin ($[\alpha]_D$ +63°) has a slightly negative rotation ($[\alpha]_D$ −5°) which is enhanced in magnitude in its acetyl and methyl derivatives. Hydrolysis followed by chromatographic analysis showed galactose (70%) to be the principal constituent of this resistant material but xylose (*ca.* 7%) was present also, the amount being in reasonable agreement with the pentose content of the original polysaccharide (Part I, *loc. cit.*). Methylation proved to be difficult, and the product of the highest methoxyl content (OMe, 40·9%) was prepared from a specimen of the acetate by deacetylation and methylation. Hydrolysis of this methylated substance and analysis by quantitative paper chromatography gave tetramethyl galactopyranose (1 part), 2 : 4 : 6-trimethyl galactose (2·3 parts), and 2 : 4-dimethyl galactose (1·5 parts). Separation on a powdered cellulose column (Hough, Jones, and Wadman, *J.*, 1949, 2511) gave crystalline trimethyl D-xylopyranose (*ca.* 0·4 part), tetramethyl galactopyranose (*ca.* 1 part), 2 : 4 : 6-trimethyl galactose (*ca.* 2·8 parts), 2 : 4-dimethyl galactose (*ca.* 1·3 parts), and momomethyl galactoses (*ca.* 1 part). In view of the scale of this experiment it is not claimed that the relative proportions indicated are more than rough approximations. The figures for the last three sugars listed are indeed almost certainly too low since certain mixed fractions containing these were not analysed. The trimethyl xylopyranose may have been derived from a contaminating xylan such as that found

in the red seaweed, *Rhodymenia palmata* (Barry and Dillon, *Nature*, 1940, **146**, 620), and it cannot be assumed that a D-xylopyranose end-group is present in the galactan.

The tetramethyl galactopyranose had $[\alpha]_D$ −43° and gave a crystalline aniline derivative, m. p. 185—187° depressed to 175° on admixture with tetramethyl D-galactopyranose anilide. On recrystallisation almost pure tetramethyl L-galactopyranose anilide was obtained, and from the specific rotation of the original syrup it is concluded that *ca.* 70% of the tetramethyl galactose in the mixture was the L-form.

The trimethyl galactose which was identical with 2 : 4 : 6-trimethyl galactose on the paper chromatogram gave a crystalline aniline derivative, m. p. 165°, from which with some difficulty a specimen, m. p. 174°, was obtained which was principally 2 : 4 : 6-trimethyl L-galactose anilide. The original sugar failed to crystallise, unlike the readily crystallisable 2 : 4 : 6-trimethyl D-galactose, and from the observed specific rotation ($[\alpha]_D$ −43°) it was presumed to contain *ca.* 75% of the L-isomer.

The dimethyl galactose gave a good yield of 2 : 4-dimethyl D-galactose anilide, and no evidence for the presence of the L-isomer was found in this case.

The list of constituents in the mixture of methylated sugars obtained on hydrolysing the resistant fragment of carragheenin, after methylation, must be revised as follows; the relative proportions indicated being rough approximations only. Trimethyl D-xylopyranose (1 part), tetramethyl D-galactopyranose (1 part), 2 : 4 : 6-trimethyl D-galactose (2 parts), 2 : 4-dimethyl D-galactose (4 parts), tetramethyl L-galactopyranose (2 parts), and 2 : 4 : 6-trimethyl L-galactose (6 parts). The monomethyl galactoses were probably a mixture of 2-methyl and 4-methyl galactose but no structural significance is attached to this fraction at present in view of the possibilities of incomplete methylation and of demethylation during hydrolysis. Carragheenin could, therefore, be depicted as a complex structure based on a resistant backbone relatively rich in L-galactose residues, branched, as witness the high proportion of terminal groups, the branching-points being D-galactose residues linked through $C_{(1)}$, $C_{(3)}$ and $C_{(6)}$, and with intermediate D- and L-galactopyranose units linked through $C_{(1)}$ and $C_{(3)}$. To this backbone which constitutes about one-sixth of the whole molecule, chains of 1 : 3-linked D-galactose units having sulphate groups on $C_{(4)}$ would be attached to those residues appearing as terminal groups in the exposed resistant skeleton. There is indeed no reason to deny that both D- and L-galactose units could co-exist in the same polysaccharide as in the snail galactogen of Bell and Baldwin (*J.*, 1941, 125), and it may be recalled that D- and L-galactose derivatives have been isolated on several occasions from seaweed polysaccharides, *e.g.*, from *Porphyra laciniata* (Tollens, *Ber.*, 1901, **34**, 1422), *Porphyra tenera* (Miwa, *Jap. J. Bot.*, 1940, **11**, 42), and agar (Jones and Peat, *J.*, 1942, 225; Forbes and Percival, *ibid.*, p. 1844). The possibility still exists, however, that carragheenin is a mixture, and that the resistant residue is a separate polysaccharide; we have not yet succeeded in giving a definite answer to this question.

Experimental.

The Partial Hydrolysis of Carragheenin.—The *Chondrus crispus* polysaccharide (hot extract) prepared as described in Part I (*loc. cit.*) (9 g.) in oxalic acid (400 c.c.; 0·1N.) containing potassium oxalate (0·05M.) was heated at 100° for 4 hours in an atmosphere of nitrogen. On being poured into ethanol (800 c.c.) a precipitate (0·8 g.) was obtained which was removed, and no further precipitate appeared on dilution with a further quantity of ethanol (2 l.). After neutralisation with barium carbonate, filtration, and concentration at 30°/15 mm., a glass (7·8 g.) was obtained which contained 5% of free galactose (estimated as the phenylmethylhydrazone). When the glass, dissolved in water, was dialysed for five days, the water being changed daily against distilled water, using a cellophane membrane, and all the solutions evaporated, 81% of the material was recovered. Free sulphate ions were absent; $[\alpha]_D^{14\cdot5}$ +22·5 (*c*, 1·8 in water) (Found: SO_4, 17·9%).

Methylation Experiments.—The glass (7·3 g.) was methylated as described for maltose (Haworth and Leitch, *J.*, 1919, 809), with methyl sulphate (114 c.c.) and sodium hydroxide (111 c.c.; 30%), the final reaction mixture being just alkaline. Extraction with chloroform gave a brown syrup (0·7 g.) which was chiefly tetramethyl methylgalactopyranoside, as shown by hydrolysis and analysis on the paper chromatogram (this substance arose from the free galactose which contaminated the starting material). The extracted solution was then poured into ethanol (4 volumes) and the precipitated salts removed by filtration after being kept overnight. The solution was then evaporated to dryness, and the product dried with alcohol–benzene and extracted with ethanol to give a yellow glass (5 g.) (Found: OMe, 23·2%).

This product was treated with thallous ethoxide (15 g.) in ethanol–benzene (1 : 1), and the thallium complex precipitated on " Hyflo " Super Cel by evaporation at 40°/15 mm. The powdered material was boiled with methyl iodide (150 g.) until no longer alkaline. After filtration the residue was exhaustively extracted with acetone, and the filtrate and washings on evaporation gave a brown glass (3·5 g.); $[\alpha]_D^{19}$ +21° (*c*, 1·0 in chloroform) (Found: OMe, 41·6; SO_4, 20·4%).

Hydrolysis and Glycoside Formation.—The methylated glass (3 g.) in oxalic acid (120 c.c.; 0·5N.) was hydrolysed at 100° for 18 hours in the presence of chloroform (20 c.c.) to prevent undue access of air. Neutralisation with barium carbonate, filtration, and evaporation gave a product which was boiled with methanolic hydrogen chloride (70 c.c.; 3%). The brown solution was then neutralised with silver carbonate and evaporated to give a brown syrup (2·5 g.).

Fractionation of the Mixture of Glycosides.—The syrup (2·5 g.) in water (30 c.c.) together with a little barium carbonate was extracted with light petroleum (b. p. 40—60°) in a continuous extractor (Brown and Jones, *J.*, 1947, 1344) to give, after 19 hours 0·48 g., and after 24 hours a further 0·03 g. of syrup; these products were combined (1). The solution was then transferred to the apparatus for extraction by solvents heavier than water, and extracted with chloroform to give (2), 1·11 g. after 46 hours, 0·02 g. only of this being extracted in the last 24 hours. The aqueous solution was evaporated to give a mixture of a syrup and inorganic material (3).

Examination of Fraction (1).—Fraction (1) was distilled at 90—95° (bath temp.)/0·01 mm. to give a liquid (0·34 g.), n_D^{14} 1·4538 (1*a*), and a smaller fraction (0·06 g.), n_D^{14} 1·4570 (1 *b*) (Found : OMe, 50·5%).

Fraction (1*a*) in sulphuric acid (50 c.c.; N.) was heated at 100° for 6 hours. After neutralisation with barium carbonate and the usual treatment a reducing product was obtained (0·3 g.); $[\alpha]_D^{13}$ +67° (*c*, 3·3 in water) (Found : OMe, 50·0. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). This substance (0·1 g.) in ethanol (5 c.c.) was treated with aniline (0·05 g.) at 80° for 1·5 hours. On evaporation (to 3 c.c.) and cooling, there were obtained the characteristic needles of tetramethyl galactopyranose anilide, m. p. 176° depressed to 157° on admixture with 2 : 4 : 6-trimethyl D-galactose anilide (m. p. 175°), raised to 188° on admixture with tetramethyl D-galactopyranose anilide. Recrystallisation from ethanol gave a product, m. p. 186—188° not depressed on admixture with tetramethyl D-galactopyranose anilide (m. p. 193°).

Fraction (1*b*) on hydrolysis and examination on the paper chromatogram gave a spot R_G 0·67 identical with a control of 2 : 4 : 6-trimethyl galactose; a minute trace of tetramethyl galactopyranose was also detected.

Examination of Fraction (2).—Fraction (2) on distillation gave the following sub-fractions: (2*a*) (0·11 g.), b. p. 92—97° (bath temp.)/0·07 mm., n_D^{15} 1·4575 (Found : OMe, 51·0%); (2*b*) (0·33 g.), b. p. 97—107° (bath temp.)/0·01 mm., n_D^{15} 1·4602 (Found : OMe, 51·3%); (2*c*) (0·13 g.), b. p. 107—140° (bath temp.)/0·01 mm., n_D^{15} 1·4697 (Found : OMe, 44·8%); and (2*d*) residue (0·5 g.).

Fraction (2*a*) in sulphuric acid (10 c.c.; N.) for 4 hours at 100° changed from $[\alpha]_D^{18}$ +58° ⟶ +75°. Conversion into the aniline derivative gave an impure product (m. p. 130—146°, raised to 160° on being washed with ether–ethanol). The crude derivative, dissolved in light petroleum–chloroform (6 : 1), was passed through a short column of activated alumina (5 × 0·7 cm.). The first volume of eluate on evaporation gave a product (5 mg.), m. p. 172° unchanged on admixture with 2 : 4 : 6-trimethyl D-galactose anilide (m. p. 175°). The mother-liquors obtained from the recrystallisations and washing processes were evaporated and hydrolysed with sulphuric acid (0·1N.) at 100° for 3 hours, and, after neutralisation, the free sugars (0·23 g.) were extracted by using ether. Examination on the paper chromatogram by direct comparison with an authentic specimen showed 2 : 4 : 6-trimethyl galactose to be the only reducing component.

Fraction (2*b*) showed $[\alpha]_D^{17}$ +69° (*c*, 3·3 in N-sulphuric acid) after 5 hours at 100°. The isolated sugar gave an aniline derivative, m. p. 168° not depressed on admixture with 2 : 4 : 6-trimethyl D-galactose anilide but depressed to 145° on admixture with 2 : 3 : 4-trimethyl D-galactose anilide (m. p. 169°).

Fraction (2*c*) had $[\alpha]_D^{18}$ +66° (*c*, 1·3 in N-sulphuric acid) after 4 hours at 100°. Examination of the isolated sugar on the paper chromatogram showed 2 : 4 : 6-trimethyl galactose to be the only component.

The residue (2*d*) was extracted with hot water, the solution evaporated, and the syrupy residue (0·08 g.) hydrolysed and examined on the chromatogram. 2 : 6-Dimethyl galactose (R_G 0·51) and 2 : 4 : 6-trimethyl galactose (R_G 0·67) were found to be present.

Examination of Fraction (3).—This mixture on extraction with cold ethanol and evaporation of the extracts gave a pale-yellow glass (0·55 g.) (Found : OMe, 16·8%). This still contained inorganic material. Hydrolysis and examination on the paper chromatogram showed the principal sugar component to be 2 : 6-dimethyl galactose.

Isolation of a Resistant Fraction from Carragheenin.—Dry, powdered, carragheenin (hot extract from *Gigartina*) (9·8 g.) was shaken vigorously for 24 hours at room temperature with methanolic hydrogen chloride (920 c.c.; 1%). The residue was removed on a sintered-glass filter and washed with dry methanol (3·7 g.). This product was then purified by four further treatments with methanolic hydrogen chloride, and, after being washed with methanol, dissolved in warm water in the presence of barium carbonate and centrifuged; concentration and precipitation in ethanol gave a substance (*R*) (2·1 g.) which had $[\alpha]_D^{14}$ −5° (*c*, 1·3 in water), +19·4° after 18 hours at 100° in 0·5N-sulphuric acid [Found : SO_4, 1·5; H_2O (microZerewitinow), 9%]. Analysis of the hydrolysate by the paper chromatogram (Flood, Hirst, and Jones, *J.*, 1948, 1678) gave galactose 71 and xylose 7%.

The methanolic solution was neutralised with silver carbonate, filtered, and treated with hydrogen sulphide, the excess of which was removed by aeration; the solution was neutralised with barium carbonate, filtered, and evaporated to dryness. The non-reducing colourless glass (7·1 g.) had $[\alpha]_D^{18}$ +13°, rising to +31° on hydrolysis with sulphuric acid (0·5N.) [Found : SO_4, 28·8; OMe, 16·6; galactose (phenylmethylhydrazone) 40%].

No sugar other than galactose could be detected in the hydrolysed material, and a search for organic acids using ion-exchange resins proved abortive. Barium dimethyl disulphate was isolated by passing a solution of the glass through " Zeo-Karb H.1. " and the acid solution was then percolated through a

column of " Deacidite B." The neutral solution so obtained gave on evaporation a glass which on hydrolysis gave 72·5% galactose by quantitative chromatogram. When the " Deacidite B " column was treated with barium hydroxide (0·2N.) and the excess removed by carbon dioxide, after filtration, evaporation, and extraction with aqueous ethanol crystalline barium methyl sulphate was isolated.

The Acetylation and Methylation of the Resistant Fraction.—The substance *R* (10 g.) was acetylated by a modification of the method of Pacsu and Mullen (*J. Amer. Chem. Soc.*, 1941, **63**, 1487). After dissolution in water, addition of pyridine and dehydration by distillation with the addition of more pyridine, the product was gelatinised by addition of pyridine (240 c.c.). Acetic anhydride (190 c.c.) was then added with vigorous mechanical stirring and after being stirred overnight the mixture was heated at 60° for 30 minutes. A small quantity of insoluble gel was removed by centrifugation, and the remainder poured into ice-water and the precipitate (*A*) filtered off and washed with water. The insoluble residue did not dissolve on further acetylation at 70° and was also precipitated in ice-water (*B*). (*A*) (8·2 g.) had $[\alpha]_D^{18}$ −39° (*c*, 1·0 in acetone) (Found : Ac, 36·5). (*B*) (0·9 g.) (Found : Ac, 21·0; ash, 4%). (*A*) in chloroform (200 c.c.) was treated with light petroleum (b. p. 40—60°; 60 c.c.) to give (*a*) (1·44 g.; Ac, 36·0%); with a further 30 c.c. (*b*) 3·55 g. (Ac, 39·1%); and on evaporation (*c*) 2·24 g., $[\alpha]_D^{15}$ −56° (*c*, 2·2 in chloroform) (Found : Ac, 40·6%).

A mixture of (*b*) and (*c*) (4·5 g.) was simultaneously deacetylated and methylated as for glycogen acetate (Haworth and Percival, *J.*, 1932, 2279). The solution obtained after treatment with methyl sulphate and sodium hydroxide was extracted with chloroform, and the product obtained on evaporation of the chloroform extracts was methylated three times with methyl iodide and silver oxide to give a product (1·25 g.), $[\alpha]_D^{15}$ −34·5° (*c*, 2·0 in chloroform) (Found : OMe, 25·1%).

The aqueous solution was neutralised with acetic acid and evaporated to crystallisation. After filtration the aqueous solution was dialysed, evaporated, and remethylated with sodium hydroxide and methyl sulphate, and the product obtained by extraction with chloroform was added to the material extracted from the crystalline residue by ethanol; the mixture was methylated twice with methyl iodide and silver oxide to give further partly methylated material (0·8 g.), $[\alpha]_D^{15}$ −49° (*c*, 2·0 in chloroform) (Found : OMe, 32·2%). This product was added to that obtained by the original chloroform extraction, and the mixture remethylated with methyl sulphate and sodium hydroxide. After dialysis a glass (*X*) (1·37 g.), $[\alpha]_D^{15}$ −50° in chloroform, was obtained (Found : OMe, 40·9%).

Attempts to obtain a better yield of the methylated product by direct methylation with sodium hydroxide and methyl sulphate under nitrogen were unsuccessful.

By using the thallium method (Hirst and Jones, *J.*, 1938, 502) the polysaccharide (3 g.) on treatment with thallous hydroxide (45 c.c.; N.) gave a thallium complex (8·1 g.) which was filtered off and dried by treatment with ethanol–benzene and removal of the solvents at 25—30° (diminished pressure). The product was ground and boiled for 7 days with methyl iodide containing 10% methanol. Extraction of the neutral solid with water, after removal of methyl iodide by distillation, gave a product (2·4 g.) (Found : OMe, 17·7%). This was dissolved in dry methanol and thallium ethoxide (1·25 c.c.) in benzene (25 c.c.) was added at 0°. After filtration the solid was dried and boiled for 4 days in methyl iodide containing methanol (5%). Extraction with boiling acetone gave a pale yellow glass (*Y*) (Found : OMe, 35·3%). Five treatments with methyl iodide and silver oxide failed to increase the methoxyl content.

The Hydrolysis and Analysis of the Methylated Fragment.—The glass (*X*) (1·37 g.), in methanolic hydrogen chloride (80 c.c.; 2%), was boiled for 36 hours at 70°. After neutralisation with silver carbonate a brown syrup (1·34 g.) was obtained. This product in sulphuric acid (25 c.c.; N.) was heated at 100° for 7·5 hours in an atmosphere of nitrogen, and the solution neutralised with barium carbonate, filtered, and evaporated at 30°/15 mm. to give a golden syrup (1·11 g.). Analysis on the paper chromatogram using alkaline iodine (Chanda, *et al.*, *J.*, 1950, 1289) gave the following sugars in the approximate molecular proportions indicated: tetramethyl galactopyranose (1 part), 2 : 4 : 6 trimethyl galactose (2·4 parts), 2 : 4-dimethyl galactose (1·5 parts), as well as trimethyl xylopyranose and monomethyl galactose(s) in smaller proportion.

Separation on the cellulose column. The sugars (0·86 g.) were separated on a column of powdered cellulose (35 × 3·25 cm.) by using a 7 : 3-mixture of petroleum (b. p. 100—120°) and butanol (water saturated) (Hough, Jones, and Wadman, *loc. cit.*). Samples (*ca.* 4 c.c.) were collected at intervals of 6 minutes. After the collection of 500 tubes the column was allowed to drain, and a 4 : 1-mixture of butanol (water-saturated) and ethanol was used for elution, the time interval being adjusted gradually to 24 minutes. The contents of every tenth tube were evaporated and examined on the paper chromatogram, the papers being developed with saturated aniline oxalate solution. The following fractions were isolated :

I (161—220), R_G 0·95; II (321—340), R_G 0·895; III (371—500), R_G 0·88; IV (521—593), R_G 0·79; V (594—600), R_G 0·79, 0·67; VI (601—620), R_G 0·67; VII (621—639), R_G 0·67, 0·60, 0·52, 0·44; VIII (640—668), R_G 0·44; IX (669—690), R_G 0·44, 0·19; X (691—721), R_G 0·19. Tubes 722—786 showed the presence of traces of xylose and galactose.

Examination of fractions. Fraction I (0·034 g.), $[\alpha]_D^{22}$ +18° (*c*, 3·0 in water), crystallised completely; it had m. p. 89° not depressed on admixture with authentic trimethyl D-xylopyranose with which it was identical on the chromatogram.

Fraction II (0·01 g.) was a waxy substance which, on extraction with water, gave no spot on the chromatogram.

Fraction III (0·087 g.), $[\alpha]_D^{23}$ −43° (Found: OMe, 50·5. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). It was chromatographically identical with tetramethyl galactopyranose. The aniline derivative had m. p. 185—187° depressed to 174° with tetramethyl D-galactopyranose anilide. On recrystallisation the product had $[\alpha]_D^{19}$ +70° (*c*, 0·4 in acetone), m. p. 192—193° depressed to 173—176° on admixture with

tetramethyl D-galactopyranose anilide ($[\alpha]_D$ −80° in acetone) (Found : N, 4·6. Calc. for $C_{16}H_{25}O_5N$: N, 4·5%).

Fraction IV (0·036 g.), $[\alpha]_D^{22}$ +47°, was not identified.

Fraction VI (0·226 g.), $[\alpha]_D^{20}$ −43° (*c*, 2·2 in water) (Found : OMe, 40·3. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). It was chromatographically identical with 2 : 4 : 6-trimethyl galactose. This syrup (0·03 g.) gave an aniline derivative (0·012 g.), m. p. 165° {$[\alpha]_D^{15}$ +49° (initial value; *c*, 0·5 in acetone)} raised to 174° by recrystallisation from ethanol and depressed to 167° on admixture with 2 : 4 : 6-trimethyl D-galactose anilide. The recrystallised aniline derivative had $[\alpha]_D^{14}$ +65° (initial) changing to −20° (1 day) (*c*, 1·1 in acetone); 2 : 4 : 6-trimethyl D-galactose anilide has $[\alpha]_D$ −92° falling to +38° (22 hours) (Hirst and Jones, *J.*, 1939, 1482), so that the aniline derivative obtained from fraction VI contains 80—85% of the L-isomer (Found : N, 5·0; OMe, 29·5. Calc. for $C_{15}H_{23}O_5N$: N, 4·7; OMe, 31·3%).

Fraction VIII (0·103 g.), $[\alpha]_D^{21}$ +47·6 (*c*, 1·0 in water) (Found : OMe, 29·1. Calc. for $C_8H_{14}O_6$: OMe, 29·9%). The syrup (0·034 g.) gave an aniline derivative which separated from the hot ethanolic solution after 30 minutes. Cooling, filtration, and recrystallisation from ethanol gave prisms (0·019 g.), m. p. 208° not depressed on admixture with authentic 2 : 4-dimethyl D-galactose anilide (Found : N, 5·2; OMe, 20·5. Calc. for $C_{14}H_{21}O_5N$: N, 5·0; OMe, 21·9%).

Fraction X (0·068 g.), $[\alpha]_D^{23}$ +42·5° (*c*, 0·7 in water), failed to give a crystalline derivative. Examination on the paper chromatogram showed the absence of 6-methyl galactose.

Fractions V, VII, and IX were mixtures, and amounted together to 0·26 g.; total recovery 0·82 g. (95%).

Examination of methylated specimen (Y). A portion of (*Y*) was hydrolysed and the methylated sugars released estimated as before by the quantitative chromatogram to give trimethyl xylopyranose (1·3 parts), tetramethyl galactopyranose (1 part), 2 : 4 : 6-trimethyl galactose (2·6 parts), dimethyl sugars (2·7 parts), and monomethyl sugars (1·2 parts). The ratio of trimethyl to tetramethyl galactose is in good agreement with that found for (*X*).

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