20. *The Polysaccharides of Iceland Moss* (Cetraria Islandica). *Part I. Preliminary Study of the Hemicelluloses.*

By H. Granichstädten and E. G. V. Percival.

The investigation is described of a " hemicellulose " extracted from Iceland moss, hydrolysis of which yielded a mixture of sugars containing glucose (89%), galactose (8%) and mannose (3%), and a uronic acid (5%), probably *d*-glucuronic acid. Methylation and fractionation, with molecular-weight determination by the viscosity method, showed that the hemicellulose was not homogeneous, although the mean molecular weight was similar to that of lichenin. Hydrolysis of four fractions (A), (B), (C) and (D), of the methylated hemicellulose resulted in the detection of a terminal end group of *d*-galactopyranose in (A), and end-groups of both galacto- and gluco-pyranose in (B). The main fraction of each hydrolysate consisted of a mixture of trimethyl glucoses and it is thought that the hemicellulose is a mixture of polysaccharides made up chiefly of β-glucose units linked through positions 1 : 2, 1 : 3, 1 : 4 and 1 : 6, the first being a type of linkage not so far reported for glucose units

Buston and Chambers (*Biochem. J.*, 1933, **27**, 1691) have shown that Iceland moss yielded hemicelluloses (3·2%) extractable with cold sodium hydroxide solution (4%), after removal of lichenin (64%) and lichen acids (8·5%). Separation by the copper method of Norris and Preece (*ibid.*, 1930, **24**, 59) gave two hemicellulose fractions, B_1 and B_2, although the relative yields varied rather widely in different experiments. These fractions were remarkable in containing no glucose in their hydrolysis products, B_1 being made up of galactose (48%), mannose (44%) and uronic acid residues (10·7%), whereas B_2 had galactose (68%), mannose (22%) and uronic acid residues (9%). Our interest in polysaccharides containing galactose led us to study these hemicelluloses to see if there was any similarity in structure to such algal polysaccharides as agar.

Partial separation by precipitation by alcohol from a faintly acid solution of a mixture of hemicelluloses obtained by the method of Buston and Chambers (*loc. cit.*) gave two fractions : (I) (89% of the total) containing uronic anhydride (4·5%); and (II) containing 7·5% of uronic anhydride. The corresponding acetates and methylated derivatives of (I) were shown by fractional precipitation not to be of uniform composition, a fact which serves to emphasise the difficulty of isolating the so-called hemicelluloses in a pure state. On account of this complication and the large quantity of solvents necessary for the extraction of these substances it has only been practicable so far to make a preliminary study of (I).

The uronic acid content is lower than that of either of the hemicelluloses (B_1) and (B_2) of Buston and Chambers, but the most striking difference is the high proportion of *d*-glucose (89%) in the mixture of sugars in the hydrolysis products of the polysaccharide, galactose amounting to but 7·6% and mannose 3%. Furthermore the uronic acid appeared to be not *d*-galacturonic acid but *d*-glucuronic acid. Such large differences in properties can be explained in a variety of ways : (*a*) unsatisfactory fractionation, (*b*) hemicelluloses present derived from the fungus as well as from the algal cell walls, (*c*) variation due to the age of the lichens investigated, which may be accentuated by the extremely slow growth period and variations in geographical or climatic conditions.

It may also be recalled that Ulander and Tollens (*Ber.*, 1906, **39**, 401) obtained glucose in quantity by the hydrolysis of *Cetraria islandica* after the removal of lichenin, and but little mannose and galactose, but showed that the hydrolysis of " Reindeer " mosses gave chiefly galactose and mannose.

The methylated hemicellulose (I) was divided arbitrarily by fractional precipitation from chloroform solution by light petroleum into four fractions, the properties of which (after remethylation in some cases) are recorded in Table I together with those of methylated lichenin.

Table I.

	OMe, %.	$[\alpha]_D^{12°}$ in $CHCl_3$.	$\eta_{sp.}^{25°}$.	*c*.	Apparent mol. wt., $K_m = 1 \times 10^{-3}$.
A	40·5	—	0·428	0·3115	28,000
B	43	+11°	0·771	0·3065	51,000
C	43	+ 2·5	0·767	0·3635	42,000
D	41	− 2	0·209	0·3440	12,000
Methylated lichenin	43	−12	0·479	0·3045	32,000

It is not claimed that the apparent molecular weights are absolute values, but they are tabulated in this form for ease of comparison (cf. Haworth, Heath, and Peat, J., 1942, 57).

The composition of the substance (I) is clearly not uniform and it is highly probable that the individual fractions must also represent mixtures; fraction (B) and (C) appear to be of higher and (D) of lower molecular

weight than methylated lichenin. In order to attempt to gain some idea of the basic composition of the hemicelluloses each fraction was subjected to methanolysis and the products were studied. The bulk of the methylated methylglycosides in every case were to be found in a middle fraction (80—90% of the total distillate), which on complete methylation and hydrolysis yielded tetramethyl glucopyranose, thus confirming the view that glucose was the predominant building unit in these hemicelluloses. The corresponding trimethyl glucose prepared by hydrolysis of this middle fraction in each case yielded crystalline 2 : 3 : 6-trimethyl glucose. Each fraction also yielded a crystalline anilide (8—18%), m. p. 162—166°, which proved to be 2 : 4 : 6-*trimethyl glucose anilide*, since it readily gave a crystalline trimethyl glucose, m. p. 124—126°, which gave tetramethyl glucopyranose on methylation and hydrolysis, a trimethyl β-methylglucoside, m. p. 71° (cf. Oldham, *J. Amer. Chem. Soc.*, 1934, **56**, 1360), and a crystalline amide, m. p. 99°, which gave a negative Weerman reaction (cf. Barker, Hirst, and Jones, J., 1938, 1696). The other possibilities it was necessary to envisage were 2 : 3 : 4- and 3 : 4 : 6-trimethyl glucoses and the method of Barker, Hirst, and Jones (*loc. cit.*) was used to estimate these. The proportion of 2 : 3 : 4-trimethyl glucose was estimated by the method of Oldham and Rutherford (*J. Amer. Chem. Soc.*, 1932, **54**, 366) and the Weerman reaction was employed to determine the amount of trimethyl glucose with a free hydroxyl group on C_2. The proportion of 2 : 3 : 6-trimethyl glucose was estimated from the magnitude of the change in rotation of the mixture in 2% methyl-alcoholic hydrogen chloride, and the proportion of 2 : 4 : 6-trimethyl glucose by difference. Great precision is not claimed for these figures, especially the latter, but they are probably correct to 5% (Table II).

TABLE II.

Trimethyl glucoses, %, in main fractions of hydrolysed methylated hemicellulose I.

	2 : 3 : 4-.	2 : 3 : 6-.	2 : 4 : 6-.	3 : 4 : 6-.
A	31	30	25 (13)	14
B	13	29	8 (6)	50
C	9	47	5 (7)	39
D	6	50	10 (13)	34

Figures in parentheses denote yields calculated from the yield of anilide.

From (A) a small quantity of tetramethyl *d*-galactopyranose anilide was isolated (3·5% as the methyl-glycoside), showing that a non-reducing terminal group of *d*-galactopyranose was present in this fraction.

From (B) a small quantity (2·5%) of a fully methylated methylglycoside was isolated. This proved to be a mixture of 2 : 3 : 4 : 6-tetramethyl *d*-glucose and 2 : 3 : 4 : 6-tetramethyl *d*-galactose as shown by the isolation of the crystalline anilides. This hemicellulose fraction must therefore have a branched-chain structure with at least two branches terminated by *d*-glucopyranose and *d*-galactopyranose units respectively.

The results of this investigation may be interpreted in at least two different ways. First, hemicellulose (I) could be a mixture of at least four polysaccharides differing mainly by reason of the different linkages between the β-*d*-glucose residues (1 : 2-, 1 : 3-, 1 : 4- or 1 : 6-), or secondly that we have a mixture of polysaccharides in each of which a mixture of linkages is present. Hitherto the linkage of glucose units in polysaccharides built up of glucose has been found to be uniform, the 1 : 4-type in cellulose, starch, glycogen and lichenin, 1 : 3 in laminarin (Barry, *Sci. Proc. Roy. Dublin Soc.*, 1939, **22**, 66), and the 1 : 6-type in the dextran produced from sucrose by *Leuconostoc Dextranicum* (Peat *et al.*, J., 1939, 581). On the other hand a mannan from yeast has been shown to be made up of one type of unit, *viz.*, mannose, linked in three different ways, 1 : 2-, 1 : 3-, and 1 : 6- (Haworth, Heath, and Peat, J., 1941, 833), and there is no valid reason to doubt the possibility that such mixed linkages could be present in the polysaccharides discussed here. The presence of glucopyranose units linked through C_1 and C_2 has not been previously reported, but the above results show that almost as many are linked in this way as through C_1 and C_4.

EXPERIMENTAL.

Isolation and Purification of the Hemicelluloses.—Iceland moss (1 kg., dry weight) containing 15% of moisture was twice extracted with 1·5% sodium carbonate solution (10 l.) and twenty times with boiling water for 4 hours (15 l. each time) until the extracts on evaporation and hydrolysis were non-reducing to Fehling's solution. The residue (366 g.), dried at 70°/12 mm., was twice extracted at room temperature with 4% sodium hydroxide solution (16·1 l.), and as much as possible of the liquor separated by filtration through muslin. To a solution thus obtained (8·3 l.), after acidification with acetic acid, alcohol (9·2 l.) was added, and the brown jelly separated in a centrifuge. The material was then dissolved in 4% sodium hydroxide solution (5·14 l.) and centrifuged; Fehling's solution (1·28 l.), followed by alcohol (5 l.), was added, and the precipitated copper complex collected on muslin. This was then treated with 2N-hydrochloric acid (1 l.), and the hemicelluloses again precipitated with alcohol (2·5 l.). The product was dissolved in hot dilute sodium hydroxide solution (4·8 l.) and acidified with acetic acid. By the addition of alcohol (3440 c.c.) the bulk (89%) of the hemicelluloses (I) was precipitated and removed in a centrifuge. The addition of more alcohol (2·5 l.) precipitated the remainder (II). The bulk of the material was washed twice and kept moist, with alcohol; a portion for direct investigation was washed with alcohol and ether and dried over phosphoric oxide in a vacuum.

Hemicellulose (I) (Found: ash, 0·6%; uronic acid, 4·95%; pentose, nil). (II) (Found: uronic acid, 8·5%; pentose, nil).

Hydrolysis of (I).—(I) (4·00 g.) was heated with 3·7% sulphuric acid (100 c.c.) for 3·5 hours. Insoluble matter (0·663 g.) was removed, and the filtrate neutralised with barium carbonate, heated, and evaporated to dryness at 45°/12 mm. The resulting viscous syrup in water (8 c.c.) was poured into alcohol (2·2 l.), and the resulting flocculum collected on a Gooch filter (0·3281 g.). The alcoholic filtrate gave a glass (2·72 g.) on evaporation.

The Estimation of Artificial Mixtures of Mannose, Glucose, and Galactose.—Mannose (0·052 g.) and glucose (0·376 g.)

in water (15 c.c.) and alcohol (15 c.c.) were treated with phenylmethylhydrazine (0·75 g.) and acetic acid (1·5 c.c.). No precipitate resulted.

Galactose (0·038 g.), mannose (0·048 g.) and glucose (0·632 g.) in water (22 c.c.) and alcohol (22 c.c.) were treated with phenylmethylhydrazine (1·1 c.c.) and acetic acid (2·2 c.c.). After 48 hours at 10° the precipitate was washed with alcohol (3 c.c.) and dried at 105°. The yield of galactosephenylmethylhydrazone corresponded to 3% of galactose instead of 5·2%.

Mannose (0·400 g.) and galactose (0·712 g.) in water (11 c.c.) were treated with phenylhydrazine (0·8 c.c.), acetic acid (0·8 c.c.) and water (2 c.c.). After 48 hours at 0° the precipitate was washed with ice-water (200 c.c.), alcohol (10 c.c.) and ether (10 c.c.) and dried at 105°. The yield of mannosephenylhydrazone corresponded to 34·5% of mannose instead of 35·6%.

Mannose (0·035 g.), galactose (0·028 g.) and glucose (0·49 g.) in water (60 c.c.) were treated with phenylhydrazine (1·25 c.c.), acetic acid (1·25 c.c.) with sodium acetate (1 g.) and sodium bisulphite (0·05 g.) for 1 hour at 160°. After 24 hours the precipitate was collected and washed with dilute acetic acid and water. The yield of osazone corresponded to 55% of the weight of glucose present.

The Estimation of the Hexoses in the Hydrolysis Products of (I).—This was carried out according to the above methods, the appropriate factors being applied [Found: Galactose, 7·6; mannose, 3·0; glucose 96% (mean of two determinations)].

Hydrolysis of (I) *with* 15% *Sulphuric Acid.*—(I) (7·3 g.) was treated at 100° for 24 hours with sulphuric acid (125 c.c., 15%). The insoluble residue (0·43 g.) was removed, and the solution neutralised with the calculated quantity of barium hydroxide solution in the presence of charcoal. After evaporation to 5 c.c., and precipitation in alcohol (2·5 l.), a white flocculent precipitate (0·95 g.) was separated in a centrifuge, and was washed with alcohol and ether and dried in a vacuum over phosphoric oxide; $[\alpha]_D^{18°}$ +13° in water (*c*, 1·1). Oxidation with 6N-nitric acid at 60° gave no mucic acid. Treatment with *p*-bromophenylhydrazine gave a yellow precipitate characteristic of *d*-glucuronic acid (cf. Hirst and Jones, J., 1938, 1179, who record $[\alpha]_D^{18°}$ +15° for the barium *d*-glucuronate).

Acetylation of (I).—(I) (10 g.) which had been kept in alcohol was suspended in pyridine (150 c.c.); acetic anhydride (100 c.c.) was added in five batches at 10-minute intervals with mechanical stirring, and the mixture maintained for 3 hours at 100°. The solution was set aside for 48 hours and filtered through glass wool; the precipitate produced by addition of a large volume of water was washed in running water for 24 hours (yield 14 g.). The product had $[\alpha]_D^{18°}$ *ca.* −10° in chloroform [Found: $CH_3{\cdot}CO$, 43·5%; $\eta_{sp.}^{25°}$ 0·436 (*c*, 0·3000); M_v = 36,400, K_m being taken as 9×10^{-4}]. For lichenin acetate $\eta_{sp.}^{25°}$ 0·514 (*c*, 0·3525), M_v = 36,400. M_v represents apparent molecular weight only. The acetate was non-homogeneous and was fractionated after methylation.

Deacetylation and Methylation.—Hemicellulose (I) acetate containing moisture (70%) (dry weight 30 g.), dissolved in boiling acetone (1·2 l.), was treated with methyl sulphate (600 c.c.) and sodium hydroxide solution (1500 c.c., 30%) with vigorous stirring in the usual way at 40—45°. The temperature was then raised slowly to remove acetone and the solution was finally heated at 75—80° for 1 hour. The brown solid admixed with sodium sulphate was collected hot and washed with boiling water. All the available acetate (110 g.) was thus twice methylated; two batches were then united and again subjected to two methylations. Yield, 64 g.

Fractionation of Methylated Hemicellulose (I).—The above product was dissolved in chloroform (1500 c.c.), dried with anhydrous sodium sulphate, and filtered. Light petroleum (8·8 l.) (b. p. 60—80°) was added with vigorous stirring to yield (A) (14·06 g.), which was separated in a centrifuge. Light petroleum (1·5 l.) precipitated (B) (15·76 g.), and a further 2·3 l. (C) (15·46 g.). The solution on evaporation yielded (E) (18·72 g.). (A), (C) and (E) were remethylated. The properties are recorded in Table I.

Hydrolysis of (A) *and Fractionation.*—(A) (8·4 g.) was heated for two 15-hour periods at 70° with methyl-alcoholic hydrogen chloride (170 c.c., 5%). Neutralisation with silver carbonate and removal of solvent yielded a syrup (7 g.), which was separated into two fractions by distillation at 0·05 mm.; these were redistilled from a flask fitted with a vacuum-jacketed column [except for fractions (IV) and (V)]. In this way five fractions were obtained as follows (all the b. p.'s in this and subsequent distillations refer to bath temperatures): (A1) 0·74 g., b. p. 135°/0·04 mm., $n_D^{20°}$ 1·4526, OMe 53·5%; (A2) 1·26 g., b. p. 145—160°/0·04 mm., $n_D^{20°}$ 1·4593; (A3) 0·58 g., b. p. 168—180°/0·02 mm., $n_D^{20°}$ 1·4585; (A4) 2·16 g., b. p. 160—165°/0·02 mm., $n_D^{20°}$ 1·4591, OMe 51·2%; (A5) 0·8 g., b. p. 165—175°/0·02 mm., $n_D^{20°}$ 1·4632, OMe 47·8%.

(A1) was slowy refractionated, yielding 0·2 g., $n_D^{20°}$ 1·4511. This on hydrolysis with N-sulphuric acid at 100° for 10 hours yielded a sugar (0·165 g.), which was treated with aniline (0·08 c.c.) in alcohol (0·8 c.c.) at 80° for 2 hours. The crystals obtained were recrystallised from ether–light petroleum yield (0·024 g.); m. p. 196°, unchanged by tetramethyl *d*-galactopyranose anilide, $[\alpha]_D^{20°}$ −80° in acetone (*c*, 0·4).

Investigation of the Trimethyl Methylglycosides.—(A4) was methylated several times with methyl iodide and silver oxide. The product on hydrolysis with N-sulphuric acid and anilide formation yielded tetramethyl glucopyranose anilide in good yield, m. p. 138°, not depressed by an authentic specimen.

Fractions (A3) and (A4) (combined) were hydrolysed with N-sulphuric acid for 9 hours at 95—100°, and the sugars isolated in the usual way.

Isolation of 2 : 4 : 6-*Trimethyl Glucose.*—(A3) and (A4) (combined) were hydrolysed to give a reducing syrup (1·4 g.), which on heating with aniline (2 c.c.) and alcohol (5 c.c.) at 95° for 3 hours gave a crystalline *anilide* after several days, two crops of which (0·24 g.) were removed. The anilide after recrystallisation from alcohol had m. p. 162—166°, $[\alpha]_D^{15°}$ −113° in methyl alcohol (*c*, 1·0), constant, 24 hours. (Found: C, 60·1; H, 7·8; OMe, 30·1; N, 5·2. $C_{15}H_{23}O_5N$ requires C, 60·6; H, 7·8; OMe, 31·3; N, 4·7%).

Repeated attempts to prepare crystalline anilides from 2 : 3 : 6- and 3 : 4 : 6-trimethyl glucose were unsuccessful.

Hydrolysis for 2 hours with sulphuric acid (3%) at 95°, followed by neutralisation with barium carbonate, extraction with ether, and removal of water at 40°/15 mm., gave a sugar (60%) which crystallised spontaneously; recrystallisation from dry ether gave 2 : 4 : 6-trimethyl α-*d*-glucose, m. p. 124—126°, $[\alpha]_D^{15°}$ +109° (initial), +74° (8 hours, constant) in water (*c*, 1·0); $[\alpha]_D^{15°}$ +107° (initial), +70° (24 hours, constant) in 2% methyl-alcoholic hydrogen chloride (*c*, 1·01) (Found: C, 48·5; H, 8·1; OMe, 41·2. Calc. for $C_9H_{18}O_6$: C, 48·7; H, 8·1; OMe, 41·9%).

Three methylations with methyl iodide and silver oxide and hydrolysis gave quantitatively tetramethyl glucopyranose, m. p. 88° alone or mixed with an authentic specimen.

A small quantity of the sugar was oxidised with bromine, and the acid esterified with diazomethane; the amide obtained on treatment with ammonia had m. p. 99°, $[\alpha]_D^{15°}$ +47° in water (*c*, 0·5), and gave a negative Weerman reaction.

The sugar (0·1 g.) was acetylated with pyridine and acetic anhydride, the acetate treated with hydrogen bromide–acetic acid, and the product shaken in methanol with silver carbonate. Deacetylation gave a crystalline glucoside (0·1 g.), which after recrystallisation from light petroleum had m. p. 71°, $[\alpha]_D^{15°}$ −26° in water (*c*, 1·0) (Found: C, 50·4; H, 8·5; OMe, 51·8. Calc. for $C_{10}H_{20}O_6$: C, 50·8; H, 8·5; OMe, 52·5%).

Investigation of Position C_6.—(A4) (0·8 g.) in pyridine (1 c.c.) was treated with *p*-toluenesulphonyl chloride (1 g.) and kept at room temperature for 48 hours. Water was then added, the solution extracted with benzene, and the extract washed with dilute hydrochloric acid, aqueous sodium bicarbonate and water and dried over anhydrous sodium sulphate.

Removal of solvent gave a syrup (1·135 g.), which was heated with anhydrous sodium iodide (1·2 g.) in acetone (11 c.c.) at 100° for 2 hours in a sealed tube. The precipitated sodium *p*-toluenesulphonate (0·1752 g.) was washed with dry acetone. This indicated that 31% of the starting material was unsubstituted on C_6 (Calc. from 1·135 g. of 6-tosyl-2 : 3 : 4-trimethyl methylglucoside : 0·5647 g. of sodium *p*-toluenesulphonate). With later fractions the iodo-compound was decomposed with silver nitrate in methyl cyanide, and the silver iodide weighed.

When 3-tosyl 2 : 4 : 6-trimethyl methylgalactoside (0·1270 g.) was treated as described above, no sodium *p*-toluenesulphonate or silver iodide was obtained.

Investigation of Position C_2.—A portion of the hydrolysed fractions (A3) and (A4) (combined) was oxidised with bromine at room temperature until it was non-reducing. The solution was then repeatedly extracted with chloroform after removal of bromine, and after drying over sodium sulphate and removal of solvent lactone formation was completed at 100°/15 mm. This was followed by distillation at 125—150°/0·06 mm. to yield a lactone (0·25 g.), $n_D^{19°}$ 1·4655. Immediately after distillation the lactone was treated with methyl-alcoholic ammonia and kept at 0° for 2 days; removal of solvent gave an amide, $[\alpha]_D^{19°}$ +50·5° in methyl alcohol (*c*, 3·8). The amide (0·2715 g.) was dissolved in water (7 c.c.), standard sodium hypochlorite (4·7 c.c.) (Weerman, *Rec. Trav. chim.*, 1917, **36**, 16) added, and the mixture kept at 0° for 3 hours. Excess of hypochlorite was destroyed by sodium thiosulphate, anhydrous sodium acetate (2·7 g.) added, and then semicarbazide hydrochloride (0·54 g.) was added to the filtered solution. After 12 hours the precipitated hydrazodicarbonamide was collected, washed with water (2 c.c.), and dried over phosphoric oxide. Yield, 0·0144 g. of hydrazodicarbonamide, m. p. 256°, *i.e.*, 14% of the practical yield [75% of the theoretical yield (see below)], so 14% of (A3) and (A4) is unsubstituted on C_2.

A control experiment with *d*-gluconamide (0·2072 g.) carried out under identical conditions gave hydrazodicarbonamide, m. p. 256° (0·0958 g.), *i.e.*, 76·4% of the theoretical. This was twice repeated. 3 : 4 : 6-Trimethylgluconamide (0·1976 g.) gave hydrazodicarbonamide (0·0736 g.), *i.e.*, 75% of the theoretical.

Proportion of 2 : 3 : 6-*Trimethyl Methylglucosides in* (A).—Hydrolysed (A3) and (A4) (combined) showed $[\alpha]_D^{20°}$ +70° (initial), +39·7° (constant value after 24 hours) in 2% methyl-alcoholic hydrogen chloride (*c*, 0·4).

Authentic 2 : 3 : 6-trimethyl glucose showed $[\alpha]_D^{18°}$ +70° (initial), −32° (constant value after 24 hours) (*c*, 0·4).

3 : 4 : 6-Trimethyl glucose prepared according to Reynolds (J., 1933, 225) and Haworth, Hirst, and Panizzon (J., 1934, 154) showed $[\alpha]_D^{20°}$ +69° (initial, constant) in 2% methyl-alcoholic hydrogen chloride (*c*, 0·63).

The equilibrium rotation of 2 : 4 : 6-trimethyl glucose in the same solvent was found to be $[\alpha]_D^{15°}$ +70°, and the rotation of 2 : 3 : 4-trimethyl glucose in methanol is given as +69° (Irvine and Oldham, J., 1921, **119**, 1744). It being assumed that the presence of the other methylated glucoses does not affect the final equilibrium of 2 : 3 : 6-trimethyl glucose in methyl-alcoholic hydrogen chloride to any great extent, since the equilibrium rotations of the other three possibilities are *ca.* +70°, the proportion of 2 : 3 : 6-trimethyl glucose in the mixture concerned is 30%.

It is concluded, therefore, that about 85% of the methylglycosides obtained by the hydrolysis of (A) are trimethyl methylglucosides made up of the following trimethyl glucoses : 2 : 3 : 4- (31%), 3 : 4 : 6- (14%), 2 : 3 : 6- (30%), and 2 : 4 : 6- (by difference, 25%). The isolation of tetramethyl galactopyranose anilide shows that *d*-galactose end groups were present to the extent of about 3·5%.

Hydrolysis and Fractionation of (B).—The methylated compound (15·31 g.) was hydrolysed as described for (A) to give a mixture of glycosides (16·54 g.), $[\alpha]_D^{13°}$ +48·8° in methanol (*c*, 0·95). Distillation from an ordinary flask gave 15·52 g. of distillate, which was divided into three fractions, b. p. 93—110°/0·01 mm., 110—127°/0·01 mm., and 127—150°/0·01 mm. These were redistilled through a vacuum-jacketed column to give the following : (B1), 0·36 g., b. p. 126—141°/0·005 mm., $n_D^{12°}$ 1·4492, OMe 57·3%; (B2) 1·68 g., b. p. 141—153°/0·005 mm., $n_D^{12°}$ 1·4560; (B3), 3·24 g., b. p. 153—160°/0·005 mm., $n_D^{12°}$ 1·4622, OMe 50·4%; (B4) 7·18 g., b. p. 150—155°/0·01 mm., $n_D^{12°}$ 1·4615; (B5) 1·14 g., b. p. 158—173°/0·01 mm., $n_D^{12°}$ 1·4610; (B6) 0·90 g., b. p. 165—175°/0·01 mm., $n_D^{12°}$ 1·4654; (B7) 0·10 g., b. p. 175—195°/0·01 mm., $n_D^{12°}$ 1·4701, OMe 45·6%; (B8) 1·00 g., residue, $n_D^{12°}$ 1·4838.

Isolation of Tetramethyl Galactopyranose Anilide and Tetramethyl Glucopyranose Anilide from (B1).—On distillation (B1) gave [B1(*a*)] 0·11 g., b. p. 85—88°/0·04 mm., $n_D^{16°}$ 1·4470, $[\alpha]_D^{12°}$ +63° in water (*c*, 0·3); [B1(*b*)] 0·15 g., b. p. 95—100°/0·04 mm., $n_D^{16°}$ 1·4481, OMe 59·2%.

[B1(*a*)] was hydrolysed to give a syrup (0·07 g.), which yielded tetramethyl *d*-galactopyranose anilide on suitable treatment, m. p. 198°, not depressed by an authentic specimen.

[B1(*b*)] on treatment in a similar way gave an anilide (0·03 g.), m. p. 196°, identical with tetramethyl *d*-galactopyranose anilide (Found : C, 61·5; H, 7·9. Calc. for $C_{16}H_{25}O_5N$: C, 61·7; H, 8·1%).

On standing for some time more crystals were deposited which on recrystallisation had m. p. 136°, unchanged on admixture with tetramethyl glucopyranose anilide.

Investigation of the Trimethyl Methylglycosides.—(B3) (0·61 g.) was twice methylated with methyl iodide and silver oxide, and the product distilled to give a mobile oil (0·5 g.), b. p. 85—90°/0·01 mm., $n_D^{16°}$ 1·4472 (Found : OMe, 61·0. Calc. for $C_{11}H_{22}O_6$: OMe, 62%).

The oil was hydrolysed for 7 hours with N-sulphuric acid (15 c.c.); the sugar, $[\alpha]_D^{12°}$ +85° in water (*c*, 0·4), isolated in the usual way, crystallised on nucleation with tetramethyl glucopyranose. Anilide formation yielded an anilide, m. p. 136—138°, not depressed on admixture with tetramethyl glucopyranose anilide (Found : C, 61·7; H, 8·2; OMe, 39·6; N, 4·4. Calc. for $C_{16}H_{25}O_5N$: C, 61·7; H, 8·1; OMe, 39·9; N, 4·5%).

Hydrolysis and Fractionation of (C).—Methylated (C) (17·09 g.) was hydrolysed with methyl-alcoholic hydrogen chloride (4%) for 30 hours and with methyl-alcoholic hydrogen chloride (5%) for 45 hours to yield a syrup (15·49 g.), $[\alpha]_D^{11°}$ +96·5° in methanol (*c*, 0·58). Distillation yielded 13·7 g. of glycosides, which were separated into three fractions, b. p. 115—130°/0·03 mm., b. p. 130—145°/0·01 mm., and b. p. 145—180°/0·01 mm., each of which was distilled from a flask through a vacuum-jacketed column as before to yield the following fractions : (C1) 1·20 g., b. p. 132—145°/0·01 mm., $n_D^{17°}$ 1·4538; (C2) 1·79 g., b. p. 145—155°/0·01 mm., $n_D^{17°}$ 1·4580, OMe 51·7%; (C3) 3·64 g., b. p. 155—165°/0·01 mm., $n_D^{17°}$ 1·4598; (C4) 3·93 g., b. p. 145—175°/0·01 mm., $n_D^{17°}$ 1·4600; (C5), 1·28 g., b, p. 160—170°/0·03 mm., $n_D^{15°}$ 1·4621; (C6) 0·37 g., b. p. 170—195°/0·03 mm., $n_D^{15°}$ 1·4621, OMe 14·78%; (C7) 1·03 g., residue, $n_D^{16°}$ 1·4729.

Fraction (C1). Fraction (C1) was redistilled through the fractionating column and gave [C1(*a*)] 0·44 g., b. p. 142—146°/0·02 mm., $n_D^{15°}$ 1·4558, OMe 51·0%, and [C1(*b*)] 0·76 g., residue.

[C1(*a*)] was redistilled from a small flask, the first two drops having b. p. 92°/0·02 mm., $n_D^{14°}$ 1·4577, OMe 49·5%, indicating the absence of a fully methylated end group. [C1(*a*)] was also heated with 0·1N-potassium hydroxide at 100° for 2·5 hours, but no alkali was used up, showing the absence of an ester group.

[C1(*a*)] was then methylated three times with Purdie's reagents; the product, hydrolysed, and subjected to anilide formation, gave tetramethyl glucopyranose anilide, m. p. and mixed m. p. 136°, in good yield.

Investigation of the Trimethyl Methylglucosides. Fraction (C4).—(C4) was fully methylated by Purdie's method and distillation gave an oil, b. p. 100°/0·03 mm., $n_D^{16°}$ 1·4466, OMe 61·0%, which on hydrolysis and treatment with aniline gave tetramethyl glucopyranose anilide in good yield, m. p. 138°, not depressed by an authentic specimen.

Hydrolysis and Fractionation of (D).—Methylated (D) (17·14 g.) was treated thrice with boiling methyl-alcoholic hydrogen chloride for 45 hours, and the product worked up as before to yield a mixture of glycosides (18·47 g.), $[\alpha]_D^{13°}$

+94° in methanol (*c*, 0·4). Distillation yielded 16·66 g., which were redistilled to give three fractions, b. p. 118—130°/0·03 mm., 132—142°/0·02 mm., 140—150°/0·01 mm. Redistillation as previously described gave 7 fractions: (D1) 0·28 g., b. p. 130—155°/0·03 mm., $n_D^{14°}$ 1·4560, OMe 51·6%; (D2) 3·10 g., b. p. 150—160°/0·03 mm., $n_D^{14°}$ 1·4601; (D3) 6·68 g., b. p. 159—167°/0·03 mm., $n_D^{14°}$ 1·4609; OMe 49·0%; (D4) 3·64 g., b. p. 159—167°/0·03 mm., $n_D^{16°}$ 1·4618; (D5) 1·75 g., b. p. 167—175°/0·03 mm., $n_D^{15°}$ 1·4639; (D6) 0·20 g., b. p. 175—195°/0·03 mm., $n_D^{15°}$ 1·4680, OMe 48·3%; (D7) 0·76 g., residue, $n_D^{14°}$ 1·4828.

Investigation of the Trimethyl Methylglucosides.—A portion (0·25 g.) of (D2) was completely methylated with methyl iodide and silver oxide and on distillation gave an oil, $n_D^{16°}$ 1·4466, OMe 61·0%, which gave tetramethyl glucopyranose anilide in good yield on hydrolysis and suitable treatment, m. p. 138°, not depressed by an authentic specimen.

The hydrolysis and examination of fractions of trimethyl methylglucosides from (B), (C), and (D) were carried out as for (A); the results are in Tables III, IV, V, and VI.

TABLE III.

Investigation of Position C_2.

(1).	(2).	(3).	(4).	(5).
A3 + A4	+50·5°	0·2715	0·0144	14
B3	+54	0·1276	0·0252	52
		0·2082	0·0373	48
C3 + C4	+44	0·1750	0·0259	39
D3 + D4	+45	0·2349	0·0298	34
D3 + D4	—	0·2314	0·0305	35

(1) Fraction number.
(2) $[\alpha]_D^{19°}$ of amide in methanol.
(3) Weight of amide, g.
(4) Yield, g., of hydrazodicarbonamide from (3).
(5) % with unsubstituted OH on C_2.

TABLE IV.

Investigation of Position C_6.

(1).	(2).	(3).	(4).	(5).	(6).
A4	1·135	0·1752	—	—	31
B4	0·980	0·064	—	—	13
B4	—	—	0·6071	0·0514	12
C4	—	—	1·203	0·0718	8·8
D4	—	—	1·03	0·0391	5·6

(1) Fraction number.
(2) Wt. of tosyl derivative, g.
(3) Yield, g., of sodium *p*-toluenesulphonate from (2).
(4) Weight of *p*-toluenesulphonate after treatment with sodium iodide, g.
(5) Yield of silver iodide from (4), g.
(6) % with unsubstituted OH on C_6.

TABLE V.

Investigation of Position C_4.

(1).	(2).	(3).	(4).	(5).	(6).
A3 + A4	—	—	—	+39·7°	30
B3	54	+37·5°	+46·2°	—	29
B4	—	—	—	+40	30
C3	66	+11·5	+33·3	—	50
C4 + C5	—	—	—	+22·5	46·5
D3	95	−24	—	—	—
D3 + D4	—	—	—	+18·7	50

(1) Fraction number.
(2) Crystalline trimethyl glucose, %.
(3) Equilibrium $[\alpha]_D^{18°}$ in methanol–HCl (2%) of crystals.
(4) Equilibrium $[\alpha]_D^{18°}$ in methanol–HCl of residual syrup after removal of crystals.
(5) Equilibrium $[\alpha]_D^{16°}$ of hydrolysed fraction in same solvent.
(6) 2 : 3 : 6-Trimethyl glucose, %.

TABLE VI.

Yields of 2 : 4 : 6-*Trimethyl Glucose Anilide.*

(1).	(2).	(3).	(4).
A3 + A4	1·4	0·24	17
B4 + B5	1·0	0·09	9
C4 + C5	1·64	0·146	9
D3 + D4	1·44	0·256	18

(1) Fraction number.
(2) Weight of hydrolysed fraction, g.
(3) Yield of anilide, g.
(4) % Yield of anilide.

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