LXXII. POLYSACCHARIDES SYNTHESISED BY MICRO-ORGANISMS.

I. THE MOLECULAR STRUCTURE OF MANNOCAROLOSE PRODUCED FROM GLUCOSE BY *PENICILLIUM* *CHARLESII* G. SMITH.

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It has recently been shown that, using glucose as the sole source of carbon, a number of hitherto undescribed metabolic products are formed when *Penicillium Charlesii* G. Smith is grown on Czapek-Dox solution of the sugar [Clutterbuck *et al.*, 1934]. Among the products are two new polysaccharides one of which consists only of mannose residues and the other of galactose residues. In addition, six hitherto unknown organic acids are found. The present communication describes the investigation of the molecular constitution of the polymannose to which is given the name *mannocarolose*. This is a white, amorphous, hygroscopic powder, $[\alpha]_{5780} + 66^\circ$, readily soluble in water and forming an acetate which is soluble only in organic solvents. Repeated fractional precipitation of the polysaccharide and removal of ash by electrodialysis yielded a product which was essentially homogeneous and on hydrolysis this gave a yield of 97 % of mannose, isolated as the phenylhydrazone. The molecular weight of the acetate was calculated by the use of the Staudinger factor from the viscosity measurements and corresponded to 9 mannose units in the molecule. This value is in agreement with that determined by the estimation of the mean iodine number. The same value is also found by a direct determination or gravimetric assay of the end group of the methylated polysaccharide. The latter yielded, on hydrolysis, 13.4 % of tetramethyl-methylmannoside which corresponds to a chain length of 8-9 mannose units. It is of considerable interest to record the general agreement of all three methods of determining the molecular size of the polymannose.

The precise form in which mannose is combined in the polysaccharide whether as pyranose or furanose, and also the particular mode of linking of adjacent units of the hexose were investigated in the following way. Methylated mannocarolose was completely hydrolysed, the hydrolysis products converted into the glycosides, and a quantitative analysis of the products revealed the presence of 75 % of a trimethyl-methylmannoside (I), 13.4 % of tetramethyl-methylmannoside, together with another fraction (10 %) which was identified as mainly a dimethyl-methylmannoside. On complete methylation and hydrolysis, the trimethyl- and dimethyl-mannosides yielded 2:3:4:6-tetramethylmannopyranose (III) which was characterised as its crystalline anilide.

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The trimethylmannose fraction was identified as a crystalline 2:3:4-trimethylmannopyranose (II), M.P. 102–103°, $[\alpha]_{5780}^{21} + 7^\circ$ (equilibrium value) which is now described for the first time. The positions of the methyl groups were determined and the experimental evidence for the allocation of the 2:3:4-positions is here given. The trimethyl sugar gave, on oxidation, 2,3:4-trimethylmannopyranolactone (IV) which was distilled; its hydrolysis curve in aqueous solution and the rate of lactonisation of the corresponding acid were characteristic of a δ -lactone of the mannose series. It gave on further methylation a quantitative yield of tetramethylmannopyranolactone characterised as its crystalline phenylhydrazide. It was evident therefore that the hydroxyl group at position 4 in the trimethyl-sugar must have been methylated. This was shown conclusively by the degradative oxidation of the trimethylmannonolactone (IV) with nitric acid, which yielded *d*-arabotrimethoxyglutaric acid (V) and *i*-dimethoxysuccinic acid (VI) both of which were characterised as their crystalline methylamides [Goodyear and Haworth, 1927]. The isolation of the former of these products proves that the methylated positions in the trimethylmannose

must be at hydroxyls 2, 3, 4; that position 5 must be engaged in ring-formation; and that the terminal position 6 is to be represented as a free hydroxyl group liberated from the methylated polysaccharide by fission during hydrolysis. It is evident therefore that mannocarulose is constituted by the linking of manno-

pyranose units at positions 1 and 6 and that the form of mutual linking of successive units of mannose characterises the structure of the polysaccharide. This mode of union of hexose units differs fundamentally from that found in starch and cellulose in which the glucose residues are united at positions 1 and 4.

In the original hydrolysis of methylated mannocarolose it was noted that about 10 % consisted of a dimethyl-sugar which on glycoside formation was identified as 2:3-dimethyl-methylmannoside in the following way. It yielded on further methylation 2:3:4:6-tetramethyl-methylmannopyranoside identified by hydrolysis and isolation of the crystalline anilide. The 2:3-dimethylmannose gave on oxidation a γ -lactone which was further degraded by oxidation with nitric acid and yielded only crystalline *i*-dimethoxysuccinic acid, characterised also as its crystalline methylamide.

In the hydrolysis products of methylated mannocarolose this 2:3-dimethylmannose occurs to the extent of 10 % corresponding very nearly with the amount of the 2:3:4:6-tetramethylmannose which was isolated as the non-reducing terminal group. It should be noted that the polysaccharide reduces alkaline hypiodite and gives an iodine number corresponding to the presence of one reducing group in the chain length of 9 mannose residues. The formulation can hardly be represented therefore by a continuous loop with a tetramethylmannose projection as a side-chain. The absence of reduction to Fehling's solution is in contrast to the hypiodite reaction. The occurrence of one dimethyl residue of the 9 which otherwise have their quota of methyl groups in the methylated polysaccharide may be significant in other directions, but at the moment we incline to the view that inasmuch as it is not possible to ensure complete homogeneity of methylated mannocarolose by a procedure corresponding to that of crystallisation it seems inevitable that any not quite completely methylated portion may account for the occurrence of this quantity of dimethylmannose. The formulation (A) is subject to this modification, and although the stereochemical form is represented as that of α -mannose it should be clearly understood that this is not based on anything more definite at the moment than the comparatively high dextrorotation (+66°) of the polysaccharide.

EXPERIMENTAL.

The crude mixture of mannocarolose and galactocarolose (*i.e.* the polygalactose) was prepared by the method described by Clutterbuck *et al.* [1934]. The yields and properties of batches on Czapek-Dox medium and on Raulin-Thom medium were consistent with those previously described. Moreover by the method of fractionation it was shown that the crude polysaccharide mixture obtained from the Czapek-Dox medium contained mannocarolose and galactocarolose identical in properties with, but different in amount from, those obtained from Raulin-Thom medium as was previously suggested [Clutterbuck *et al.*, 1934, p. 103]. About equal amounts of mannocarolose and galactocarolose were isolated from the polysaccharide mixture from the Czapek-Dox medium, whereas with the Raulin-Thom medium the proportions were 30 % and 70 % respectively.

A sample of mannocarolose, prepared as previously described, was subjected to rigorous purification to attain homogeneity. The last portions (1 %) of ash were difficult to remove by alcohol precipitations, but all traces were removed by electrodialysis during 18 hours. The product had the following properties: it was a white hygroscopic powder, $[\alpha]_{5780}^{21} + 66^\circ$ (*c*, 3.3) in water. (Found: C, 44.8; H, 6.8 %. $(C_6H_{10}O_5)_x$ requires C, 44.4; H, 6.2 %.) Its solution was neutral to

litmus and did not reduce Fehling's solution. After acid hydrolysis it gave mannosephenylhydrazone in 97 % yield and crystalline *d*-mannose in 84 % yield. The latter compound was crystallised from glacial acetic acid and the mother-liquors yielded mannosephenylhydrazone (12 %). Mean iodine number [Bergmann and Machemer, 1930], 14.3, corresponding to a molecular weight of 1400.

Mannocarolose acetate.

Mannocarolose (20 g.) was acetylated as previously described [Clutterbuck *et al.*, 1934]. The mannocarolose acetate (OAc, 46.1 %) was a fine white powder, easily soluble in chloroform and acetone, slightly soluble in ethyl alcohol and insoluble in methyl alcohol, ether and light petroleum. It was separated into four fractions by the graded addition of light petroleum to a 25 % chloroform solution. Each fraction showed identical properties. M.P. 150–160°; $[\alpha]_{5780}^{20} + 53^{\circ}$ in acetone (*c*, 1.6), $[\alpha]_{5780}^{20} + 66^{\circ}$ in chloroform (*c*, 0.84). Ash, 0.07 %. (Found: C, 50.1; H, 5.8; OAc, 46.0 %. $(C_{12}H_{16}O_8)_x$ requires C, 50.0; H, 5.6; OAc, 44.8 %.) The mean iodine number was 8.0, corresponding to a molecular weight of 2500.

Viscosity measurements were carried out on each fraction and the values obtained were identical. The molecular weight calculated by the use of the Staudinger factor from the viscosity measurements was 2584, corresponding to 9 mannose units in the molecule, a value which supports that calculated from the mean iodine number of the original polysaccharide and similar to that determined by the gravimetric assay of the end-group of the methylated polysaccharide.

Methylated mannocarolose.

(1) *By direct methylation of mannocarolose.* In a typical experiment mannocarolose (5 g.) was dissolved in water (20 ml.) and methylated in the usual manner by the gradual addition of sodium hydroxide and dimethyl sulphate during 5 hours. After 30 minutes acetone (100 ml.) was added. The temperature was maintained at 35–40° during the addition of the reagents, after which it was raised to 80–90° until all the acetone was removed (30 minutes).

Partly methylated mannocarolose was obtained as a light brown gum on the surface of the hot solution, and it redissolved on cooling. The temperature was raised and the methylated material was separated and dissolved in chloroform. The solution was dried over anhydrous magnesium sulphate and cleared by centrifuging. The chloroform was removed by distillation and the resulting thin syrup poured into light petroleum (B.P. 40–50°). The methylated product (5 g.) was precipitated as a fine cream-coloured powder. $[\alpha]_{5780}^{20} + 80^{\circ}$ in chloroform (*c*, 1.0). (Found: OMe, 36.2 %.)

The product (5 g.) was re-methylated in acetone solution (50 ml.) at 40° with dimethyl sulphate and sodium hydroxide over a period of 2 hours. Before raising the temperature to 90° for the final heating the excess alkali was carefully neutralised and the solution diluted, since it was observed that the product was appreciably soluble in hot alkali. The methylated product separated as a brown gum and was fractionally precipitated as before from chloroform solution giving a light-brown powder (yield 5.4 g.). $[\alpha]_{5780}^{20} + 96^{\circ}$ in chloroform (*c*, 1.2). (Found: OMe, 41.0 %.)

The product (5 g.) was now dissolved in methyl iodide (50 ml.) containing dry methyl alcohol (2 ml.), and re-methylated by heating gently for 4 hours with silver oxide (10 g.) which was added in 2 g. lots. The product was extracted with boiling chloroform, which was removed by distillation. On pouring the residual thin syrup into light petroleum (B.P. 40–50°) the methylated compound was

precipitated as a light brown gum, prolonged trituration of which with renewed amounts of light petroleum (B.P. 40–50°) converted it into a cream-coloured powder. M.P. 95–100°. Yield 5.4 g. $[\alpha]_{5780}^{21} + 102^\circ$ (c, 1.4) in chloroform. (Found: OMe, 44.4 %.) Further methylation by either method failed to alter the methoxyl content or the specific rotation.

(2) *By methylation of mannocarolose acetate.* Mannocarolose acetate (15 g.) was dissolved in acetone (200 ml.) and methylated at 45° by the simultaneous addition of dimethyl sulphate (140 ml.) and 30 % sodium hydroxide (290 ml.) in the usual manner. Acetone was added from time to time to retain the methylated product in solution. After the addition of the reagents (90 minutes) the temperature was raised to 80° for 30 minutes. The excess alkali was neutralised with dilute sulphuric acid and the cold solution poured slowly into ethyl alcohol (1.5 l.) and kept overnight. The separated sodium sulphate was filtered off and extracted with alcohol. The combined alcoholic solution and extracts were concentrated *in vacuo* to a viscid syrup which was dissolved in water (20 ml.) and re-methylated with dimethyl sulphate (100 ml.) and 30 % sodium hydroxide (210 ml.) in the presence of acetone at 35–40°. The product was isolated as previously described as a fine light brown powder. Yield, 10 g. $[\alpha]_{5780}^{18} + 80^\circ$ (c, 2.0) in chloroform. (Found: OMe, 36.1 %.)

The above product was re-methylated twice with silver oxide and methyl iodide and the resulting almost completely methylated mannocarolose was obtained in the form of a cream-coloured powder. Yield, 10.5 g. M.P. 95–100°; $[\alpha]_{5780}^{18} + 102^\circ$ in chloroform (c, 2.0). (Found: OMe, 44.2 %.) It was observed that the specific rotations of the partially methylated fractions gave a good indication of their methoxyl contents.

Fractionation of methylated mannocarolose.

The different preparations of methylated mannocarolose having $[\alpha]_{5780}^{21} + 102^\circ$ in chloroform and methoxyl content, 44.4 %, were mixed (20 g.) and separated into three fractions as follows. The combined material (20 g.) was boiled for 20 minutes with ether (200 ml.), which was decanted, and then for a further 10 minutes with fresh ether (200 ml.). A gummy residue (A) remained undissolved. To the mixed ether solutions was added light petroleum (B.P. 40–50°), (400 ml.) with vigorous stirring. An oily precipitate (B) separated and the clear mother-liquor was decanted after standing for an hour. Evaporation of the solvent left a gummy residue (C). Each fraction was obtained as a cream-coloured powder by trituration with light petroleum (B.P. 40–50°) and had the following properties:

Fraction	Yield (g.)	% OMe	$[\alpha]_{5780}^{20}$ in chloroform	Apparent mol. wt. by viscosity	Mol. wt. (Rast)
A	6.2	44.3	+102°	1960	2010
B	9.8	44.5	+102.5°	1960	1926
C	3.8	44.1	+101°	1960	2000

These results indicate that fully methylated mannocarolose prepared either by method 1 or 2 is fairly homogeneous. This compound was readily soluble in cold water, acetone, chloroform, methyl and ethyl alcohols, slightly soluble in ether and insoluble in hot water and light petroleum. (Found: C, 53.1; H, 7.8; OMe, 44.4 %. $(C_9H_{16}O_5)_x$ requires C, 52.9; H, 7.9; OMe, 45.6 %.)

Hydrolysis of methylated mannocarulose.

Methylated mannocarulose is comparatively stable towards acid. It was unaffected by 8 % methyl alcoholic hydrogen chloride even after boiling for several hours, but was completely hydrolysed by fuming hydrochloric acid at 15° in 4 days, the specific rotation $[\alpha]_{5780}^{18^\circ}$ changing from +71° to +5.2° (c, 1.0). 15.3 g. were hydrolysed in fuming HCl (150 ml.) at 15° for 4 days. There was considerable darkening in colour of the solution towards the end of the reaction. The excess acid was removed by aeration and the residual solution completely neutralised by the addition of barium carbonate. The solution was filtered after the addition of a little charcoal and the filtrate and washings made up to 500 ml. This colourless solution was extracted 8 times with chloroform (250 ml. each time). The combined chloroform extracts were dried over anhydrous magnesium sulphate, filtered and the chloroform removed by distillation, giving 3.1 g. of a viscid pale yellow syrup which was strongly reducing to Fehling's solution. $[\alpha]_{5780}^{18^\circ} + 28.6^\circ$ in methyl alcohol (c, 3.1). This syrup was converted into the methylmannoside by boiling with 3 % methyl alcoholic HCl for 12 hours, the product being isolated in the usual manner. Yield, 3.1 g. of a mobile non-reducing syrup (fraction *a*), $n_D^{16.5^\circ}$, 1.4597; OMe, 56.4 %.

The chloroform-extracted aqueous solution was concentrated *in vacuo* at 40° to 100 ml. and then poured into 90 % ethyl alcohol (1 l.) and filtered. The alcoholic filtrate was evaporated to dryness and the residue extracted eight times with chloroform. This chloroform solution was combined with a chloroform extract of the solid barium chloride which had been collected by the above filtration and yielded on evaporation 12 g. of a colourless viscid syrup, $n_D^{17^\circ}$, 1.4740; $[\alpha]_{5780}^{18^\circ} + 30^\circ$ (in MeOH). This syrup was converted into the methylmannosides in the usual manner. There were thus obtained 12 g. of a non-reducing colourless mobile liquid (fraction *b*), $n_D^{16.5^\circ}$, 1.4640. (Found: OMe, 51.91 %.)

Further prolonged extraction with chloroform of the alcohol and barium chloride residues gave fraction *c* (0.8 g.) as a very viscid syrup, $[\alpha]_{5780}^{20^\circ} + 27^\circ$ in methyl alcohol (c, 1.5), which was also converted into the methylmannoside. Yield, 0.8 g., $n_D^{16^\circ}$, 1.4735. (Found: OMe, 42.5 %.)

Fractional distillation of the methylated mannosides (fractions a, b, and c.)

Fraction *b* (12.0 g.) which consisted mainly of trimethyl-methylmannoside was distilled *in vacuo* from a Claisen flask and gave the following sub-fractions:

Sub-fraction	Pressure (mm. Hg)	Bath temperature	Yield (g.)	$n_D^{17^\circ}$
<i>b</i> ₁	0.12	120–125°	2.0	1.4595
<i>b</i> ₂	0.12	125–135°	6.0	1.4620
<i>b</i> ₃	0.12	135–160°	2.6	1.4650
<i>b</i> ₄	0.12	160–170°	0.8	1.4735

Sub-fraction *b*₁ was now mixed with fraction *a* (total weight, 5.1 g.) and distilled from a Claisen flask with a Widmer flask as a receiver for the first fraction, and gave the following sub-fractions:

Sub-fraction	Pressure (mm. Hg)	Bath temperature	Yield (g.)	$n_D^{17^\circ}$
<i>a</i> ₁	0.09	115–125°	4.0	1.4585
<i>a</i> ₁	0.09	125–135°	0.5	1.4610
<i>a</i> ₃	0.09	—	0.5	1.4690

(Still residue)

Sub-fraction a_1 (4 g.) was now re-distilled very slowly through the Widmer column and gave the following sub-fractions:

Sub-fraction	Pressure (mm. Hg)	Bath temperature	Yield (g.)	n_D^{20}
First drop	0.09	115–118°	—	1.4535
a_4	0.09	115–120°	1.1	1.4560
a_5	0.09	120–125°	0.68	1.4560
a_6	0.09	125–128°	0.41	1.4560
a_7	0.09	128–135°	0.20	1.4565
a_8	0.09	135–150°	1.30	1.4620
a_9	—	—	0.30	1.4620
(Still residue)				

The methoxyl content of sub-fraction a_4 was 58.08 %, and sub-fractions a_4 to a_7 had distilled without effective separation of constituents. Accordingly a thin spiral fractionating column was now used, leaving a space of 2 cm. between the top of the column and the side arm. The outside of the column was surrounded by a thick layer of asbestos paper. Sub-fractions a_4 to a_7 were combined (2.3 g.) and distilled as follows:

Sub-fraction	Pressure (mm. Hg)	Bath temperature	Yield (g.)	n_D^{21}	% OMe
A_1	0.06	118–120°	1.71	1.4512	60.7
A_2	0.06	120–125°	0.30	1.4560	58.1
A_3	—	—	0.30	1.4610	51.5
(Residue)					

The amount of tetramethyl-methylmannoside in sub-fraction A_2 was 0.15 g. Thus the total yield of tetramethyl-methylmannoside from sub-fractions A_1 and A_2 was 1.86 g. To this amount must be added a predetermined 10 % correction for losses so that the total yield of tetramethyl-methylmannoside was 2.046 g. from 15.3 g. of methylated mannocarlose, corresponding to 13.4 % of tetramethyl-methylmannoside and to a chain length of 8–9 units.

Analysis and identification of the 2:3:4:6-tetramethyl-methylmannoside.

The tetramethyl-methylmannoside from sub-fraction A_1 showed $[\alpha]_{5780}^{20} + 71^\circ$ in methyl alcohol (c. 0.84), $[\alpha]_{5780}^{20} + 46^\circ$ in water (c. 1.0). (Found: C, 52.9; H, 8.4; OMe, 60.7 %. $C_{11}H_{22}O_6$ requires C, 52.8; H, 8.9; OMe, 62.0 %.)

On treatment with 2N H_2SO_4 at 100° the tetramethyl-methylmannoside (1 g.) hydrolysed completely in 4 hours, the equilibrium rotation being $[\alpha]_{5780}^{17} + 4^\circ$. The H_2SO_4 in the hydrolysate was removed by the addition of barium carbonate, the solution filtered, concentrated *in vacuo* and the products of hydrolysis isolated by extraction with chloroform: 0.9 g. of a syrup was obtained which was strongly reducing to Fehling's solution and had n_D^{18} , 1.4631. This was treated with aniline in boiling alcohol in the usual manner and gave 2:3:4:6-tetramethylmannopyranose anilide in 85 % yield. It crystallised in long needles from a mixture of alcohol and light petroleum and had m.p. 142° alone or in admixture with an authentic specimen.

Analysis and identification of 2:3:4-trimethyl-methylmannoside.

Sub-fractions b_2 , a_2 , a_8 , a_9 , A_3 , having B.P. at bath temperatures of 125–150° at 0.06–0.12 mm. and n_D , 1.4610–1.4620, were combined and re-distilled from a Claisen flask. The product distilled completely at 130–135°/0.08 mm. as a colourless mobile syrup, n_D^{17} , 1.4620; $[\alpha]_{5780}^{20} + 58^\circ$ in water (c. 1.25). (Found: C, 50.9; H, 8.8; OMe, 51.02 %. Trimethyl-methylmannoside $C_8H_{16}O_5(OMe)_3$ requires C, 50.9; H, 8.5; OMe, 52.5 %.)

Further methylation of 2:3:4-trimethyl-methylmannoside.

1 g. of re-distilled trimethyl-methylmannoside was methylated twice with silver oxide and methyl iodide in the usual manner and the product (1 g.), distilled at 0.08 mm. at a bath temperature of 115–118°, showed n_D^{21} , 1.4515, and $[\alpha]_{5780}^{20} + 42.5^\circ$ (c, 0.83). This syrup was hydrolysed at 100° with 2*N* H₂SO₄, the rotation changing from +42.5° to +3.3° in 3 hours. 2:3:4:6-Tetramethylmannopyranose was isolated from the hydrolysis solution in the usual manner and gave 2:3:4:6-tetramethylmannopyranose anilide in 95 % yield, m.p. 142°, and identical with an authentic specimen.

Hydrolysis of 2:3:4-trimethyl-methylmannoside.

4 g. of trimethyl-methylmannoside were hydrolysed with 2*N* H₂SO₄ at 100° giving an equilibrium value of $[\alpha]_{5780}^{21} + 6^\circ$ after 3 hours. The hydrolysis product, 2:3:4-trimethylmannose, was extracted in the usual manner giving 3.8 g. of a colourless syrup having n_D^{17} , 1.4735, $[\alpha]_{5780}^{21}$ in water +9° (c, 1.25) and OMe, 40.2 %. (Theoretical for C₆H₉O₃(OCH₃)₃, 41.9 %.) This syrup failed to give a crystalline anilide and gave an oily product on treatment in the usual manner with phenylhydrazine at 70° for 3 hours. The syrup crystallised on keeping in a desiccator for 3 months and was recrystallised from ether-light petroleum (b.p. 40–50°) in the form of triangular prisms, m.p. 94–96°, $[\alpha]_{5780} + 7^\circ$ (equilibrium value) in water (c, 2.0). The m.p. 102–103° was higher when the substance was freshly crystallised as is the case with tetramethylglucose. (Found: C, 48.4; H, 8.3; OMe, 40.8 %. C₆H₉O₃(OCH₃)₃ requires C, 48.6; H, 8.1; OMe, 41.9.) Mutarotation in water (c, 20.4) zero hours $[\alpha]_{5780}^{20} + 18.2^\circ$; 5 min. +15.3°; 8 min. +13.2°; 17 min. +9.1°; 65 min. +8.1°; equilibrium value +7.0°.

Oxidation of 2:3:4-trimethylmannose.

2 g. of 2:3:4-trimethylmannose were warmed at 40° with bromine in aqueous solution for 48 hours and the lactone so formed was isolated in the usual manner. It was non-reducing to Fehling's solution and distilled at bath temperature 138–140° at 0.04 mm. Yield, 1.85 g. n_D^{21} , 1.4750, OMe, 41.7 %. (Theoretical for C₆H₁₆O₆, 42.3 % OMe.) Its hydrolysis curve in aqueous solution at room temperature was followed polarimetrically and was typical for the δ-lactone of the mannose series. Zero hours $[\alpha]_{5780} 158^\circ$; 17 hours, 122°; 22 hours, 110°; 38 hours, 105°; 48 hours, 103° (constant value). This behaviour is similar to that of a trimethylmannopyranolactone.

Further methylation of 2:3:4-trimethylmannopyranolactone.

0.5 g. of 2:3:4-trimethylmannopyranolactone was methylated with silver oxide and methyl iodide. The product was isolated by extraction with chloroform and had $[\alpha]_{5780}^{20} + 154^\circ$, changing to +77° in 24 hours. This lactone was shown conclusively to be 2:3:4:6-tetramethylmannonolactone by the formation from it in quantitative yield of the characteristic crystalline phenylhydrazide, m.p. 184–185°, alone or in admixture with an authentic specimen.

Rate of lactonisation of 2:3:4-trimethylmannonic acid at room temperature.

Initial $[\alpha]_{5780} + 22^\circ$; 18 hours, +61°; 42 hours, +77°; 66 hours, +93°; 80 hours, +98° (constant value). The amounts of lactone and acid at equilibrium were approximately 43 % and 57 % respectively.

Oxidation of 2:3:4-trimethylmannopyranolactone.

2:3:4-Trimethylmannopyranolactone (1 g.) was oxidised by heating with nitric acid (sp. gr. 1.42) for 8 hours at 90°. The excess nitric acid was removed by distillation *in vacuo* with water for 6 hours and the product esterified with methyl alcoholic HCl. The esters (0.55 g.) were distilled as follows:

Fraction	Bath temperature	Pressure (mm. Hg)	Yield (g.)	n_D^{16}	$[\alpha]_{5780}^{21}$ in methyl alcohol
I	120–125°	0.06	0.31	1.4400	–9°
II	125–135°	0.06	0.13	1.4440	–22°
III	135–150°	0.06	0.10	1.4500	—

On nucleation with methyl *i*-dimethoxysuccinate, fraction I crystallised almost completely. The crystals after being drained on a tile and recrystallised from ether-light petroleum (B.P. 40–50°) had M.P. 68° alone or mixed with an authentic specimen of inactive methyl dimethoxysuccinate. Yield 0.1 g.

The non-crystalline portions of fractions I and II were treated with methylamine in dry methyl alcohol to form the methylamides. After keeping overnight, crystals in the form of long needles (0.07 g.) separated and were recrystallised from a mixture of ethyl acetate and ether; M.P. 210° alone or in admixture with an authentic specimen of inactive dimethoxysuccinomethylamide. The mother-liquors from the preparation of the methylamide were concentrated and crystallised completely (0.15 g.), M.P. 160–165°. Fractional crystallisation from ethyl acetate-ether gave 0.07 g. pure crystals, M.P. 171°, $[\alpha]_{5780} + 58^\circ$ in water (*c*, 0.56). This compound was shown to be *d*-arabotrimethoxyglutaromethylamide. It was found that an effective and less tedious separation of inactive dimethoxysuccinomethylamide from *d*-arabotrimethoxyglutaromethylamide could be effected by fractional sublimation of a mixture of the two substances either at ordinary pressures or *in vacuo*, the latter substance subliming at a much lower temperature than the former.

Analysis and identification of 2:3-dimethyl-methylmannoside.

Fraction *c* and sub-fractions a_3 , b_3 and b_4 were combined and redistilled at a pressure of 0.08 mm. The fraction distilling with a bath temperature of 155–160° and weighing 1.5 g. had n_D^{17} , 1.4735; $[\alpha]_{5780}^{21} + 64^\circ$ (*c*, 1.25 in water). (Found: C, 49.4; H, 8.5; OMe, 41.1 %. Dimethyl-methylmannoside ($C_6H_9O_3(OCH_3)_3$) requires C, 48.6; H, 8.1; OMe, 41.9 %.) It was found by the procedure given below that the fraction consists mainly of 2:3-dimethyl-methylmannoside. On further methylation with methyl iodide and silver oxide, 2:3:4:6-tetramethyl-methylmannopyranoside was obtained, B.P. 120°/0.09 mm., n_D^{21} , 1.4515. It gave 2:3:4:6-tetramethylmannopyranose anilide, M.P. and mixed M.P. 142° in 95 % yield. The 2:3-dimethyl-methylmannopyranoside gave, after hydrolysis with 2*N* H_2SO_4 at 100° for 3 hours, 2:3-dimethylmannose, $[\alpha]_{5780}^{21} + 16^\circ$ in water (*c*, 1.2). (Found: OMe, 29.4 %. Theoretical for $C_6H_{10}O_4(OCH_3)_2$, 29.8 %.) It gave an oily osazone on treatment with phenylhydrazine. On oxidation of 0.8 g. of the 2:3-dimethylmannose with bromine at 40° for 24 hours it gave mainly 2:3-dimethylmannofuranolactone (0.6 g.), $[\alpha]_{5780}^{18} + 106^\circ \rightarrow +66^\circ$ in 14 days. This lactone on further oxidation with nitric acid (sp. gr. 1.42) yielded only *i*-dimethoxysuccinic acid, M.P. 68° (0.15 g.) characterised by the formation of its methylamide, M.P. 210°.

SUMMARY.

The molecular structure of mannocarolose, a polysaccharide produced from glucose by *Penicillium Charlesii* G. Smith, has been investigated. On acid hydrolysis mannocarolose gives *d*-mannose as the sole hydrolytic product.

Mannocarolose gives a methyl derivative which, on subsequent hydrolysis followed by glycoside formation, gives a mixture of 13.4 % of 2:3:4:6-tetra-methyl-methylmannopyranoside, 75 % 2:3:4-trimethyl-methylmannopyranoside and 10 % of 2:3-dimethyl-methylmannopyranoside. Molecular weight determinations on mannocarolose and on its acetyl and methyl derivatives indicate that the chain length of mannocarolose is represented by 8–9 units of *d*-mannopyranose linked through the 1:6 positions. This is a unique molecular structure for a polymannose. Mannan A occurring in vegetable ivory has on the other hand the same structural linking as cellulose where the hexose units are joined at positions 1:4 [Klages, 1934]. Few of the naturally occurring mannans have been investigated and it is of interest that one form is present in the polysaccharide of the tubercle bacillus [Laidlaw and Dudley, 1925].

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