LXXXVI. POLYSACCHARIDES SYNTHESISED BY MICRO-ORGANISMS

III. THE MOLECULAR STRUCTURE OF GALACTOCAROLOSE PRODUCED FROM GLUCOSE BY *PENICILLIUM CHARLESII* G. SMITH

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In Part II of this series [Haworth *et al.* 1935, 1] the isolation of a new polysaccharide, varianose, from cultures of *Penicillium varians* G. Smith was described. The structure of this polysaccharide, which consists mainly of galactopyranose residues, was determined. In the above publication it was mentioned that another polysaccharide containing only galactofuranose units had been prepared from cultures of *Penicillium Charlesii* G. Smith. The present communication describes the investigation of the molecular structure of this polysaccharide to which has been given the name galactocarolose [Clutterbuck *et al.* 1934]. This substance is a white, amorphous, hygroscopic powder, very soluble in water, $[\alpha]_{5780} - 84°$, giving a neutral solution which slightly reduces Fehling's solution on boiling. Fractional precipitation of galactocarolose with alcohol from cold $N/10$ HCl solution removed much ash and there appeared to be no evidence that the polysaccharide was other than homogeneous. It was readily hydrolysed by $N/100$ HCl at 100° and gave only *d*-galactose. It formed an acetate, $[\alpha]_{5780} - 63°$ (in chloroform), and a methylated derivative, $[\alpha]_{5780} - 84°$ (in chloroform), both of which were soluble in most organic solvents and were shown to be essentially homogeneous.

The mean iodine number of galactocarolose was 13·0, a value which corresponds to mol. wt. 1538 and to a chain length of 9–10 galactose units. The same value was obtained by direct estimation of the end group of methylated galactocarolose, 12·4 % of tetramethyl methylgalactoside being obtained which amount corresponds to a minimum chain length of 9–10 galactose units. Calculation of the mol. wt. from viscosity measurements of the acetyl and methyl derivatives of galactocarolose by applying the original Staudinger factor for cellulose gave values which are twice the above. No evidence is available to show whether this Staudinger factor can be applied to polygalactose units.

Methylated galactocarolose was hydrolysed with methyl alcoholic HCl to give directly a mixture of methylated galactosides which on fractional distillation in high vacuum gave 12·4 % of tetramethyl methylgalactoside, 80 % (approx.) of trimethyl methylgalactoside and a small amount of residual gum. The tetramethyl methylgalactoside, $[\alpha]^{20°}_{5780} - 67°$ (in H_2O), was identified as 2:3:5:6-tetramethyl methylgalactofuranoside (I) in the following manner. It was

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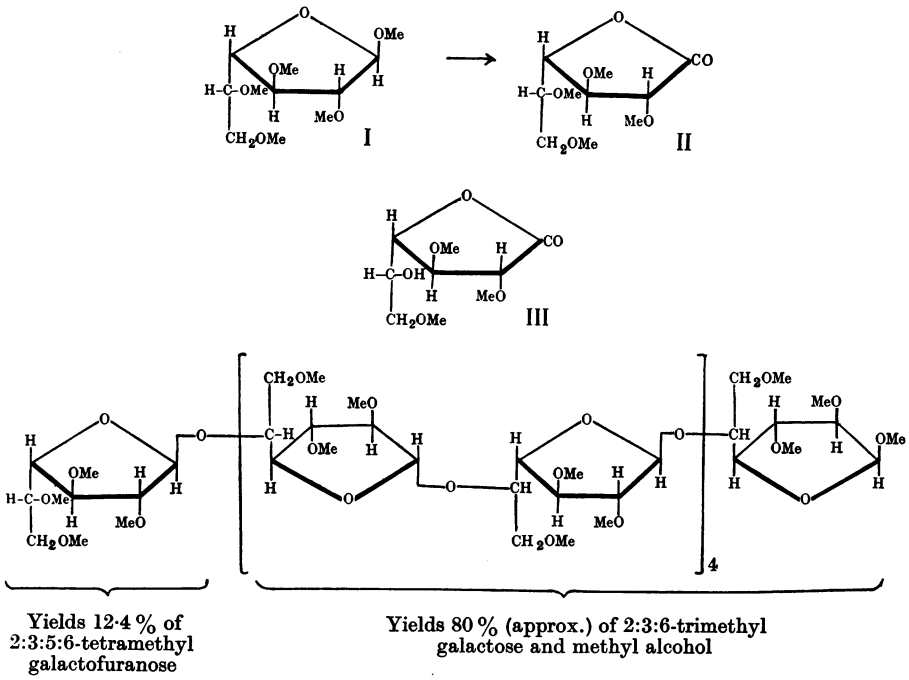
hydrolysed to 2:3:5:6-tetramethyl galactofuranose, $[\alpha]_{5780}^{20°} -23°$, and the product oxidized to the liquid 2:3:5:6-tetramethyl galactofuranolactone (II), $[\alpha]_{5780}^{20°} -33°$, changing to $-26°$ in 3 days in H_2O [cf. Haworth *et al.* 1924]. This lactone was characterized by formation of its crystalline amide, M.P. 155°, identical with an authentic specimen [cf. Humphreys *et al.* 1931].

The trimethyl methylgalactoside was readily identified as 2:3:6-trimethyl methylgalactoside since on hydrolysis it gave mainly 2:3:6-trimethyl galactopyranose $[\alpha]_{5780}^{20°} +87°$, which on oxidation gave in excellent yield crystalline 2:3:6-trimethyl galactofuranolactone (III) the latter being identical with an authentic specimen [Haworth *et al.* 1932; Haworth *et al.* 1935, 1].

The identification of 2:3:6-trimethyl galactose as the main hydrolysis product of methylated galactocarolose allows of two alternatives for the mutual linkage of the galactose residues in the polysaccharide. Inasmuch as galactocarolose is hydrolysed completely to galactose by $N/100$ HCl at 100°, and since a galactofuranose unit constitutes the non-reducing terminal residue in the chain, the polysaccharide appears to exist as a chain of 9–10 galactofuranose units mutually linked through the 1:5 positions. On rotational evidence the stereochemical form is probably that of β-galactose.



Experimental

Properties of galactocarolose. Galactocarolose was isolated in the manner previously described [Clutterbuck *et al.* 1934]. It was a white powder, insoluble in organic solvents, very soluble in water and extremely hygroscopic, reverting in a few days to a gum when exposed to moist air. Its colourless aqueous solution was neutral to litmus and reduced Fehling's solution slightly on boiling. The ash content was removed by precipitation of galactocarolose from cold $N/10$ HCl by

the slow addition of alcohol and by electrodialysis during 12 hours. (Found: C, 44·2; H, 6·2 %. $(C_6H_{10}O_5)_x$ requires C, 44·4; H, 6·2 %. $[\alpha]_{5780}-84°$ (*c*, 1·1). Mean iodine number 13·0 corresponding to a chain of 9–10 units of galactose.)

Galactocarolose was stable in boiling aqueous solution but was readily hydrolysed by *N*/100 HCl at 100° giving crystalline *d*-galactose in 90 % yield. No mannocarolose and no sugar other than galactose could be detected in the products of hydrolysis. The yield of mucic acid from galactocarolose was the same as that obtained from *d*-galactose (80 % of theoretical).

Galactocarolose acetate. The acetate [Clutterbuck *et al.* 1934] was a white powder, very soluble in chloroform, acetone and pyridine, slightly soluble in methyl and ethyl alcohols and insoluble in water and light petroleum. (Found: C, 50·3; H, 5·4; OAc, 44·4 %. $C_6H_7O_2(OCOCH_3)_3$ requires C, 50·0; H, 5·6; OAc, 44·8 %.) The acetate was separated into several fractions by the gradual addition of petroleum to a 15 % solution in chloroform. Each fraction showed $[\alpha]_{5780}-63°$ in chloroform, and gave the viscosity value $\eta_{sp}=0·25$, corresponding to mol. wt. of 4820 and to a chain of 17–18 units.

Deacetylation of galactocarolose acetate in acetone solution by *N*/10 alkali gave a hygroscopic product which was dissolved in water. Addition of cold *N* HCl and alcohol gave a white powder identical in properties, $[\alpha]^{20°}_{5780}-83°$ in water (*c*, 0·73), with the original galactocarolose.

Methylation of galactocarolose. Galactocarolose, in lots of 10 g., was dissolved in water (30 ml.) and methylated in the presence of acetone (100 ml.) by very gradual and simultaneous additions of 30 % NaOH (240 ml.) and methyl sulphate (80 ml.) over a period of 2 hours at 35–40°. The temperature was then maintained at 80° until the acetone was removed. The methylated product was dispersed throughout the hot yellow solution as a flocculent precipitate, which redissolved on cooling. The chloroform extract was evaporated and subsequent trituration of the residue with light petroleum gave a white powder. Yield, 11·3 g. OMe, 42·4 %. Two similar methylation treatments raised the methoxyl value to 43·2 %. This incompletely methylated galactocarolose was dissolved in methyl iodide (100 ml.) and methylated by the gradual addition of silver oxide (10 g.). The product was extracted with chloroform, the solution dried over anhydrous magnesium sulphate and concentrated. The residual gum was dried in a desiccator over H_2SO_4 and gave a pale yellow powder. Yield, 11·3 g. (Found: OMe, 44·6 %. $[\alpha]^{20°}_{5780}-84·8°$ in $CHCl_3$ (*c*, 1·3).)

The methylated galactocarolose (25 g.) was separated into 3 fractions by the gradual addition of light petroleum to a 6 % solution in chloroform. Each fraction showed identical properties: OMe = 44·5 %; $[\alpha]^{21°}_{5780}-82°$ (*c*, 1·3) (methyl alcohol); η_{sp} (calculated by the Staudinger factor) = 0·22 corresponding to mol. wt. 3768 and to a chain of 18–19 units. This value is about twice that obtained by the iodine number and by end group assay of the tetramethyl galactose. It was concluded that methylated galactocarolose is essentially homogeneous. (Found: C, 52·7; H, 7·8 %. $(C_9H_{16}O_5)_x$ requires C, 52·9; H, 7·9 %.)

Hydrolysis of methylated galactocarolose with methyl alcoholic HCl. Hydrolysis of methylated galactocarolose (14·8 g.) was carried out with 3 % methyl alcoholic HCl by boiling gently under a reflux; (*c*, 1·3), $[\alpha]_{5780}-82°$ (initial), $-49°$ (20 min.), $-26°$ (70 min.), $-13°$ (150 min.), $-4°$ (210 min., constant value). The product was isolated in the usual way after neutralisation with silver carbonate and extraction with chloroform. It was a colourless mobile syrup (14·7 g.) and was non-reducing showing that simultaneous hydrolysis and glycoside formation had taken place. This material was fractionally distilled by the

method described by Haworth & Machemer [1932] and the following fractions were finally isolated:

Fraction	Bath temperature 0·05 mm.	Yield g.		OMe %
I	118–120°	0·55	$n_D^{17°}$ 1·4415	61·2
II	118–120°	0·95	„ 1·4415	61·0
III	120–123°	0·22	$n_D^{22°}$ 1·4428	59·0
IV	120–126°	11·65	„ 1·4490	51·6
V	130–135°	0·51	„ 1·4520	50·0

Fractions I and II were identical and gave: C, 52·4; H, 8·8; OMe, 61·0 %. $C_{11}H_{22}O_6$ requires C, 52·8; H, 8·8; OMe, 62·0 %. This material, as indicated below, was identified as 2:3:5:6-tetramethyl methylgalactofuranoside, and showed $[\alpha]_{5780}^{20°} - 67·0°$ (*c*, 1·33 in water).

Fractions I and II were hydrolysed by heating at 100° with 0·12 *N* HCl and the specific rotation fell to −14° after 6 hours. Neutralisation of this solution with barium carbonate, filtration and concentration under diminished pressure gave, on extraction with chloroform, a colourless liquid distilling at a bath temperature of 110°/0·05 mm.; $n_D^{22°}$ 1·4488; OMe, 50·6 %. ($C_6H_8O_2(OMe)_4$ requires OMe, 52·5 %.) This reduced Fehling's solution and was identified as tetramethyl galactofuranose by the following behaviour [Haworth *et al.* 1924; Haworth & D. I. Jones unpublished]. Its yield from the galactoside was almost quantitative and confirmed the homogeneous character of fractions I and II.

Oxidation of 2:3:5:6-tetramethyl galactofuranose. The above syrup (0·8 g.) was dissolved in 20 ml. of water and oxidized with bromine (2 ml.) at 40° for 40 hours. The lactone was isolated in the usual manner (0·7 g.). It was a colourless liquid, $n_D^{22°}$ 1·4500; $[\alpha]_{5780}^{20°} - 33°$, −26° (constant value) in 3 days.

Analysis and identification of 2:3:5:6-tetramethyl galactonamide. 0·5 g. of the above tetramethyl galactonolactone was dissolved in 5 ml. of methyl alcoholic ammonia and kept at 15° for 2 days. On removal of the solvent a solid mass of crystals was deposited (0·5 g.). The compound was recrystallized from acetone/ether and had M.P. and mixed M.P. with 2:3:5:6-tetramethyl galactonamide 155°. $[\alpha]_{5780}^{20°} + 7°$ in acetone (*c*, 0·96) [Humphreys *et al.* 1931]. (Found: C, 47·7; H, 8·5; N, 5·4; OMe, 48·8 %. $C_{10}H_{21}O_6N$ requires C, 47·8; H, 8·6; N, 5·6; OMe, 49·4 %.)

The isolation of this compound characterizes the non-reducing terminal unit in galactocarolose as galactofuranose. The estimated amount of tetramethyl methylgalactofuranoside in fraction III was 0·17 g., and the total amount of tetramethyl methylgalactofuranoside from fractions I, II and III was 1·67 g. To this must be added a 10 % correction for experimental losses [Haworth & Machemer, 1932]. Thus the maximum amount from 14·8 g. of methylated galactocarolose was 12·4 % corresponding to a chain of 9–10 units.

Analysis and identification of 2:3:6-trimethyl methylgalactoside. Fraction IV had the following analysis. Found: C, 50·7; H, 8·2; OMe, 52·4 %. Trimethyl methylgalactoside $C_6H_8O_2(OMe)_4$ requires C, 50·9; H, 8·5; OMe, 52·5 %. On hydrolysis with *N* HCl at 100° (*c*, 0·9) it showed the following polarimetric changes: $[\alpha]_{5780}^{21°} + 5·1°$ (initial); +26° (15 min.); +67° (45 min.); +87° (90 min.) constant value. Thereafter 8 g. of the galactoside from fraction IV were hydrolysed and 7·8 g. of liquid 2:3:6-trimethyl galactose having $n_D^{22°} = 1·4620$; $[\alpha]_{5780}^{20°} + 87°$ in water were collected. On further methylation followed by hydrolysis and anilide formation, tetramethylgalactopyranose anilide, M.P. 186°, was isolated in 50 % yield.

Oxidation of 2:3:6-trimethyl galactose. The 2:3:6-trimethyl galactose (4 g.) was oxidized in the usual manner with bromine water at 40°. The product was

2:3:6-trimethyl galactofuranolactone (3·6 g.). (Found: C, 49·3; H, 7·4; OMe, 42·1 %. $C_9H_{16}O_6$ requires C, 49·1; H, 7·3; OMe, 42·3 %. M.P. and mixed M.P. 101°. $[\alpha]_{5780}^{20°}$ −30·6° (*c*, 1·4) in $CHCl_3$.) It formed an amide identical with an authentic specimen, M.P. 135°. Its constitution has been fully determined [Haworth *et al.* 1932; Haworth *et al.* 1935, 1].

Examination of the still residue. The total still residues were combined (1·1 g., $n_D^{22°}$, 1·4640) and distilled. A fraction, 0·51 g., distilled at bath temperature 130–135°/0·06 mm. as a pale yellow liquid, $n_D^{22°}$, 1·4520; OMe, 50·0 % and was mainly trimethyl methylgalactoside.

Methylation of a mixture of galactocarolose and mannocarolose. In an earlier paper of this series [Haworth *et al.* 1935, 2] the occurrence of galactocarolose along with mannocarolose is described and the mode of separation is somewhat tedious. A rapid method for isolating their methylated hydrolytic products has been devised. This takes advantage of the greater stability of methylated mannocarolose towards 2 % methyl alcoholic HCl. A sample (5 g.) of polysaccharide mixture, $[\alpha]_{5780}^{21°}$ −70° (containing about 10 % of mannocarolose), was methylated in the manner described. The product was a yellow powder, OMe, 44·0 %, $[\alpha]_{5780}^{20°}$ −69·7° in $CHCl_3$ (*c*, 1·3). Yield (5·5 g.).

This powder (5 g.) was dissolved in 500 ml. of 2 % methyl alcoholic HCl and boiled under a reflux for 5 hours. The solution was neutralized with Ag_2CO_3, filtered, concentrated under diminished pressure and the product extracted with chloroform. Removal of the chloroform left a colourless viscid liquid (5 g.), $[\alpha]_{5780}^{20°}$ +59·6°. Extraction of this liquid with light petroleum (40–60°) left an insoluble residue (0·48 g.) which dried in a vacuum desiccator to a yellow powder, $[\alpha]_{5780}^{20°}$ +100° in $CHCl_3$ (*c*, 1·7). This was methylated mannocarolose since it gave on hydrolysis with fuming HCl crystalline 2:3:4-trimethyl mannose which was identical with an authentic specimen.

The petroleum extracts were concentrated and the residual liquid (4·1 g.) distilled under diminished pressure at 130–145°/0·05 mm. giving a mixture of tetra- and tri-methyl methylgalactosides. $[\alpha]_{5780}^{20°}$ +7° in $CHCl_3$ (*c*, 1·3).

SUMMARY

The molecular structure of galactocarolose, a polysaccharide synthesized from glucose by *Penicillium Charlesii* G. Smith, has been investigated. On mild acid hydrolysis galactocarolose readily gives *d*-galactose as the sole product.

Galactocarolose forms a methyl derivative which, on treatment with methyl alcoholic HCl, gives a mixture of 12·4 % of 2:3:5:6-tetramethyl methylgalactofuranoside and approximately 80 % of 2:3:6-trimethyl methylgalactoside.

Molecular weight determinations of galactocarolose and its derivatives show that it has a minimum chain length of 9–10 units of β-galactofuranose mutually linked through the 1:5-positions.

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