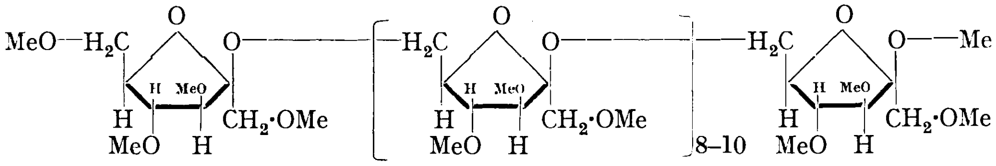
142. *Polysaccharides. Part XVII. The Constitution and Chain Length of Levan.*

By S. W. Challinor, W. N. Haworth, and E. L. Hirst.

The polysaccharide levan, obtained by Harrison, Tarr, and Hibbert (*Canadian J. Res.*, 1930, **3**, 449) by the action of *B. mesentericus* on sucrose, gives by hydrolysis an almost quantitative yield of fructose and is therefore related to the naturally occurring polysaccharide inulin. The mode of linking of its fructose units differs from that obtaining in inulin. Both carbohydrates are composed of furanose units, but whilst inulin utilises positions 1 and 2 of the fructose in effecting the mutual union of its groups, the positions participating in the linking of units in levan are those hydroxyl groups at 2 and 6 in fructose (Hibbert, Tipson, and Brauns, *ibid.*, 1931, **4**, 221). Thus the opposite terminal positions 1 and 6 are concerned in the two polysaccharides. They both display a negative rotation ($[\alpha]_D$ —36° for inulin and —47° for levan) and they are hydrolysed with similar ease.

The assay of the chain length of methylated levan (which behaves on analysis as though it were trimethyl levan) has been undertaken by the method employed in these laboratories in the cases of cellulose, starch, glycogen, inulin, etc. (J., 1932, 2270, 2277, 2368, 2372). Hydrolysis of the methylated levan revealed the presence of a terminal group recognisable as tetramethyl fructofuranose. About 10—11% of the scission product was identified as this sugar by its conversion into the crystalline amide of 2 : 3 : 4 : 6-tetramethyl fructuronic acid (compare Avery, Haworth, and Hirst, J., 1927, 2313). Special attention was paid to the possibility that levan might not be homogeneous but might consist of molecules of similar structure but of various chain lengths. All attempts, however, to separate methylated levan into fractions displaying different properties or giving different amounts of tetramethyl fructofuranose on hydrolysis, were unsuccessful. The " trimethyl levan " appeared therefore to be homogeneous. If the methylated levan be represented by the formula below, it is seen that the left-hand terminal group is the one characterised by the experimental procedure adopted and it follows that the chain length of the methylated polysaccharide consists of about 10—12 units of fructose.



Yields 1 : 3 : 4 : 6-tetramethyl fructofuranose

Yields 1 : 3 : 4-trimethyl fructofuranose

Of the occurrence of this end group as a furanose there can be no doubt, and the properties of the polysaccharide support its complete formulation as a furanose type. It should not necessarily be inferred, however, from the further conversion of the crystalline 1 : 3 : 4-trimethyl fructose into tetramethyl fructose of the furanose form that the above crystalline sugar has been isolated as a furanose. Owing to the ease of interconvertibility of a 1 : 3 : 4-trimethyl fructose into either the furanose or the pyranose modification it will depend on conditions of methylation of the free sugar whether the completely methylated tetramethyl methylfructoside appears as a furanoside or a pyranoside. Actually in the experiments recorded the furanoside was isolated.

We are unable at this stage to pronounce upon the nature of the right-hand terminal group, which has also been formulated above as a fructofuranose residue, and we have no reason at present to suspect that this is not the case. Whilst recording the iodine value of the polysaccharide, we do not connect this with the chain length. It appears somewhat anomalous and indeed the same observation applies to the viscosity measurements which give a value for the chain length which is about twice that determined by chemical assay. These factors are under investigation, not only in connexion with levan but also in so far as they concern the calculation of the molecular size of other polysaccharides, and facts have accumulated, to be published later, which may influence greatly the interpretations to be placed on these calculations.

EXPERIMENTAL.

Preparation of Trimethyl Levan.—Levan prepared from sucrose by the action of *B. mesentericus* (Harrison, Tarr, and Hibbert, *loc. cit.*) was a non-reducing white solid, $[\alpha]_{578}^{20^\circ}$ -47° in water, with properties which conformed in all respects with those recorded for levan (yield, 43 g. from 200 g. of sucrose). When levan was hydrolysed with dilute aqueous oxalic acid (0·5%), the rotation changed in the course of 1 hour from $[\alpha]_D^{18^\circ}$ -47° to -90° (the concentration in the latter case being that after hydrolysis). It would seem, therefore, that the levan used was composed entirely of fructose units (fructose has $[\alpha]_D$ -91°) (compare Hibbert and Percival, *J. Amer. Chem. Soc.*, 1930, **52**, 3995). The iodine number (Bergmann–Machemer, *Ber.*, 1930, **63**, 316, 2304) was 5·2, corresponding with an apparent molecular weight 3800. Levan (20 g. containing 7% of moisture) in water (60 ml.) was methylated with 30% aqueous potassium hydroxide (246 ml.) and methyl sulphate (86 ml.), the conditions of Hibbert, Tipson, and Brauns (*loc. cit.*) being employed. In subsequent methylations the procedure was modified, and the reaction was carried out at 55° in the presence of acetone. Under these conditions the methylated product remained in solution until precipitated by addition of water at the end of the reaction. After three treatments methylated levan was obtained as a white powder, which was washed with water and from which mineral impurities were removed by solution in chloroform. Removal of most of the solvent left a pale yellow syrup, which was boiled with dry ether, giving trimethyl levan as a white powder (yield, 85% of the theoretical) (the ether removed some mesityl oxide and other impurities), $[\alpha]_{578}^{19^\circ}$ -60° in chloroform (*c*, 2) (Found : C, 52·8; H, 7·7; OMe, 44·5. Calc. for $C_9H_{16}O_5$: C, 52·9; H, 7·9; OMe, 45·6%).

This sample of trimethyl levan was soluble in chloroform, ethyl alcohol, acetone, methyl iodide and carbon tetrachloride. It was not completely soluble in tetrachloroethylene and we were unable to measure its rotation in this solvent, in which Hibbert, Tipson, and Brauns recorded $[\alpha]_D^{23^\circ}$ -87°. Several preparations of trimethyl levan were made by the above method without variation in the properties of the final product.

Attempted Fractionation of Trimethyl Levan.—Trimethyl levan (59 g.) was dissolved in a mixture of chloroform (100 ml.) and acetone (100 ml.) and was fractionally precipitated by successive additions of light petroleum (b. p. 40—60°). After addition of a total of 4 l. of light petroleum, only a negligible amount of material remained in solution. The five precipitates weighed 51 g.; a further 3 g. ($[\alpha]_{5780}^{19^\circ}$ -59° in chloroform) which adhered mechanically to the walls of the vessels were obtained by extraction of the flasks with chloroform. This recovered material appeared to be identical with fraction III. In the following table the properties of the fractions are summarised, fraction I being the one precipitated by the first addition of light petroleum and so on in order. The abnormal m. p. and viscosity of fraction I were found to be due to its ash content, the whole of the mineral impurities of the trimethyl levan having been precipitated in this fraction.

Fraction.	Wt., g.	$[\alpha]_{5780}^{19^\circ}$ in chloroform.	M. p.*	η_{sp}.†	OMe, %.
I	7·6	-55°	115—135°	0·36	44·5
II	27·5	-60	130—132	0·11	44·3
III	11·2	-59	142—144	0·11	45·0
IV	2·8	-55	133—135	0·11	44·0
V	1·5	-58	120—122	0·11	44·4

* The m. p.'s were indefinite with previous softening and depended on conditions of heating, etc. Little significance is to be attached to the differences in m. p. of the fractions.

† In *m*-cresol at 20° (*c*, 0·02 g, in 5 ml.), using Staudinger's method. The measurements were made with the object of testing the homogeneity of the fractions. Their significance in connexion with molecular-weight determinations will be discussed later.

Hydrolysis of Trimethyl Levan, Fraction (II).—Preliminary experiments showed that by use of oxalic acid in aqueous methyl alcohol loss of trimethyl fructose by decomposition during hydrolysis was reduced to a minimum. Trimethyl levan (25 g., fraction II) was dissolved in water (250 ml.) containing oxalic acid (16 g. crystalline) and after addition of methyl alcohol (750 ml.) the solution was heated at 80° for 11 hours, during which the rotation changed from $[\alpha]_{5780}^{19^\circ}$ -50° to $-0{\cdot}5^\circ$ (constant value). After neutralisation with calcium carbonate the solution was evaporated under diminished pressure to a syrup, which was dissolved in dry ether.

Some mineral matter was thereby removed and evaporation of the ether left a pale yellow, strongly reducing syrup (27·2 g.), which was dissolved in 0·25% methyl-alcoholic hydrogen chloride (700 ml.). After 44 hours at 15° the solution no longer reduced Fehling's solution and no furfural had been produced (aniline acetate test). After neutralisation with barium carbonate the solution was evaporated (in presence of a little solid barium carbonate) at 35°/12 mm. to a mobile non-reducing syrup (27·1 g.), which gave the following fractions on distillation: (A) 14 g., b. p. 105°/0·04 mm., $n_{\mathrm{D}}^{17^\circ}$ 1·4532, OMe 50·0%; (B) 11·1 g., b. p. 108—110°/0·06 mm., $n_{\mathrm{D}}^{17^\circ}$ 1·4571, OMe 51·2%; (C) 1·0 g., b. p. 130—150°/0·05 mm., $n_{\mathrm{D}}^{17^\circ}$ 1·4672, OMe 47·5%. Still residue 0·9 g. (all temperatures are bath temperatures). Fraction (A) was distilled into a Widmer flask (bath temperature 112—115°/0·08 mm.) until 8 g. had been collected. Distillation through the Widmer column then gave (D) 2·35 g., b. p. about 80°/0·02 mm. (bath temp. 129°), $n_{\mathrm{D}}^{20^\circ}$ 1·4444, $[\alpha]_{5780}^{20^\circ}$ +79° in water (*c*, 0·9), OMe 59·7%; and (E) 0·95 g., b. p. about 85°/0·02 mm. (bath temp. 133—137°), $n_{\mathrm{D}}^{20^\circ}$ 1·4517, OMe 51·8%. The physical constants and analytical data of (D) show that it consisted almost entirely of tetramethyl methylfructoside. A further portion of (A) (4 g.) was then distilled (bath temp. 115—120°/0·05 mm.) into the Widmer flask, and the distillation from the latter continued, giving (F) 4·8 g., b. p. 82—90°/0·03 mm. (bath temp. 137—145°), $n_{\mathrm{D}}^{15^\circ}$ 1·4549, $[\alpha]_{5780}^{20^\circ}$ +70° in chloroform, OMe 52·6%. At this stage part of fraction (B) (7 g.) was distilled (bath temp. 118—120°/0·03 mm.) into the Widmer flask, and the fractionation continued, giving (G) 5·55 g., b. p. about 92°/0·03 mm. (bath temp. 142—145°), $n_{\mathrm{D}}^{15^\circ}$ 1·4557, $[\alpha]_{5780}^{20^\circ}$ +65° in chloroform, OMe 51·2%. The whole of the remaining material [(C), the remainder of (A) and (B) and the residue in the Widmer flask] was then distilled from an ordinary flask, giving (H) 7·8 g. (bath temp. 120—130°/0·03 mm.), $n_{\mathrm{D}}^{20^\circ}$ 1·4580, OMe 50·5%; (I) 1·1 g. (bath temp. 135—155°/0·05 mm.), $n_{\mathrm{D}}^{20^\circ}$ 1·4612, OMe 48·3%; (J) 1·5 g. (bath temp. 190—235°/0·1 mm.), $n_{\mathrm{D}}^{20^\circ}$ 1·4732, OMe 41·4%. A small dark-coloured resinous still residue remained. All the above distillates except (J) (which was pale yellow) were colourless oils which gave no test for furfural (aniline acetate) or unsaturated decomposition products (potassium permanganate). The total amount of tetramethyl methylfructoside [2·5 g. contained in (D) and (E)] was 10% of the weight of methylated levan subjected to hydrolysis.

Hydrolysis of Trimethyl Levan, Fractions III, IV, and V.—Fractions III (9·6 g.), IV (2·05 g.), and V (1·0 g.) of the trimethyl levan (as above) were combined for hydrolysis, the procedure being identical with that just described for fraction II. During hydrolysis the rotation changed in 10 hours from $[\alpha]_{5780}$ −54° to −0·3°. After the treatment with methyl-alcoholic hydrogen chloride 12·15 g. of non-reducing methylated methylfructosides were obtained. Furfural was absent. The preliminary distillation gave (A′) 6·97 g. (bath temperature 98—104°/0·04 mm.), $n_{\mathrm{D}}^{17^\circ}$ 1·4540, OMe 51·4%; (B′) 3·56 g. (bath temp. 106—113°/0·04 mm.), $n_{\mathrm{D}}^{19^\circ}$ 1·4576; OMe 49·8%; (C′) 0·98 g. (bath temp. 122—135°/0·07 mm.), $n_{\mathrm{D}}^{18^\circ}$ 1·4644. All of (A′) was distilled into the Widmer flask and fractionated through the column, giving (D′) 1·0 g., b. p. about 80°/0·04 mm. (bath temp. 130—136°), $n_{\mathrm{D}}^{18^\circ}$ 1·4459 (Found: C, 52·1; H, 8·9; OMe, 57·3. Calc. for $C_{11}H_{22}O_6$: C, 52·8; H, 8·8; OMe, 62·0%) (this fraction contained about 15—20% of trimethyl methylfructoside); (E′) 1·58 g., b. p. *ca.* 86—90°/0·04 mm. (bath temp. 138—142°), $n_{\mathrm{D}}^{18^\circ}$ 1·4518; (F′) 0·56 g., b. p. 86°/0·1 mm. (bath temp. 155—165°), $n_{\mathrm{D}}^{18^\circ}$ 1·4544, OMe 47·7%. Some of (B′) was then distilled into the Widmer flask, and the distillation from the latter continued. Nothing distilled below 145° (bath temp.), the next fraction being (G′) 2·65 g., b. p. *ca.* 82°/0·01 mm. (bath temp. 145—155°), $n_{\mathrm{D}}^{18^\circ}$ 1·4559, OMe 51·6%. At this stage, all tetramethyl methylfructoside having been removed, the distillation was stopped, the residue in the Widmer flask was combined with (C′) and the rest of (B′), and the mixture distilled from an ordinary flask, giving (H′) 2·76 g. (bath temp. 114—125°/0·35 mm.), $n_{\mathrm{D}}^{22^\circ}$ 1·4567, OMe 51·1; (I′) 0·12 g. (bath temp. 120—140°/0·05 mm.), $n_{\mathrm{D}}^{22^\circ}$ 1·4600, OMe 49·7%; (J′) 1·22 g. (bath temp. 140—210°/0·05 mm.), $n_{\mathrm{D}}^{22^\circ}$ 1·4640, OMe 46·2%. All the above distillates were free from furfural or reducing impurities. Fractions (D′) and (E′) together contained about 1·0 g. of tetramethyl methylfructoside, *i.e.*, 10% of the weight of material subjected to hydrolysis.

Characterisation of the Distillates.—(a) 1 : 3 : 4-*Trimethyl methylfructofuranoside.* Each of the above distillates having refractive indices, b. p.'s, and methoxyl contents corresponding with a trimethyl methylhexoside gave on hydrolysis crystalline 1 : 3 : 4-trimethyl fructose. The observations with fraction (H) were typical (Found: C, 51·1; H, 8·7; OMe, 50·5. Calc. for $C_{10}H_{20}O_6$: C, 50·8; H, 8·5; OMe, 52·6%). On hydrolysis at 80° with 0·25% hydrochloric acid (130 ml.) fraction (H) (6·5 g.) showed a change in specific rotation from $[\alpha]_{5780}$ +7° (in dilute hydrochloric acid) to −50° (constant value reached in 60 minutes). After neutralisation with silver carbonate the product was extracted with chloroform, giving 1 : 3 : 4-trimethyl fructose as a solid crystalline mass, m. p. (after recrystallisation from ether) 75°, alone or when mixed with an authentic specimen prepared by Hibbert, Tipson, and Brauns. $[\alpha]_{5780}^{18^\circ}$ $-52°$ (equilibrium value in water; *c*, 5·0) (Found : C, 48·6; H, 8·1; OMe, 41·0. Calc. for $C_9H_{18}O_6$: C, 48·7; H, 8·2; OMe, 41·8%).

On methylation by silver oxide and methyl iodide 1 : 3 : 4-trimethyl fructose gave tetramethyl methylfructoside (yield, almost quantitative), b. p. 95°/0·10 mm. $[\alpha]_{5780}^{20^\circ}$ $-1°$ in water (*c*, 0·5) (Found : OMe, 61·0. Calc. : OMe, 62·0%). This was a non-reducing mobile syrup, without action upon neutral potassium permanganate, and on hydrolysis at 80° for 4 hours with *N*/10-hydrochloric acid it gave a product which had $[\alpha]_{5780}^{20^\circ}$ $+31°$ (equilibrium value) and appeared to be mainly tetramethyl fructofuranose.

(b) 1 : 3 : 4 : 6-*Tetramethyl methylfructofuranoside*. The identity of the tetramethyl methylfructofuranoside (fractions D and D′) was proved by its conversion (in yield similar to that previously recorded for authentic tetramethyl fructofuranose) into the crystalline amide of 2 : 3 : 4 : 6-tetramethyl fructuronic acid, m. p. 99°, alone or when mixed with an authentic specimen (for details see Avery, Haworth, and Hirst, *loc. cit.*).

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UNIVERSITY OF BIRMINGHAM, EDGBASTON.

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