345. *Polysaccharides. Part XV. The Molecular Structure of Inulin.*

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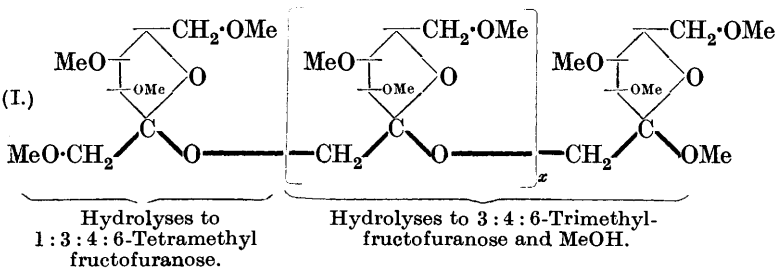
IN previous papers of this series it has been shown that evidence concerning the molecular structure of polysaccharides can be obtained by hydrolysing their fully methylated derivatives. The polysaccharides hitherto examined by this method have had a common feature in that they were derivatives of glucose and yielded on hydrolysis the extremely stable substances tetramethyl glucopyranose and 2 : 3 : 6-trimethyl glucopyranose. It is now shown that the same method of enquiry is applicable also to methylated inulin, which on hydrolysis gives labile fructofuranose derivatives. Inasmuch as methylated inulin gives 3 : 4 : 6-trimethyl fructofuranose accompanied by 1 : 3 : 4 : 6-tetramethyl fructofuranose to the extent of 3·7%, it is concluded that methylated inulin is composed of continuous chains of methylated fructofuranose residues united through positions 1 and 2 of the fructofuranose (compare Haworth and Learner, J., 1928, 619).

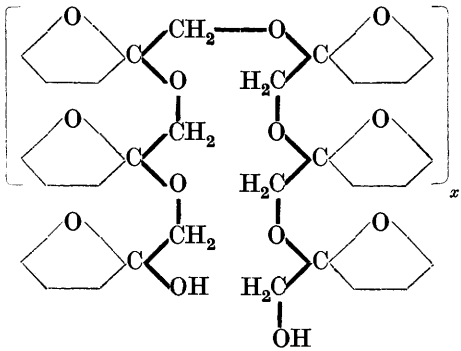
(I.)



The isolation of tetramethyl fructofuranose demonstrates clearly that the macromolecules are not in the form of large rings and establishes the nature of one of the terminal groups of the chain. Under the experimental conditions adopted, it seems extremely unlikely that appreciable degradation of the inulin molecule had taken place during the transformation to the methylated derivative. The inulin macromolecule may be pictured, therefore, as consisting of a chain of fructofuranose units united as shown in (II) and having a minimum average length of 30 fructofuranose residues. From the evidence given, the nature of one of the terminal groups may be deduced and whilst the character of the other is not immediately derivable from the present experiments the instability of inulin in the presence of alkali and the fact that it is not possible to prepare a sample of inulin free from action on Fehling's solution (Drew and Haworth, J., 1928, 2670) are strong indications that the other ter-

minal residue possesses a free reducing group (II). On this view the molecular weight of free inulin is about 5000, in striking general agreement with the value suggested by Drew and Haworth (*loc. cit.*) as a result of molecular-weight determinations made ebullioscopically on freshly prepared aqueous solutions of inulin.

(II.)



In the preparation of methylated inulin advantage was taken of improved methods for acetylation and methylation (Haworth and Streight, *Helv. Chim. Acta*, 1932, **15**, 609). Precautions were taken to carry out all reactions under the mildest possible conditions and in addition exhaustive tests were applied both to the acetate and to the methylated derivative as a check upon their essential homogeneity. In no case was it found possible to separate the acetate or the methylated derivative into fractions with different properties. This is of particular significance in the inulin series, where degradation is known to be accompanied by marked changes in physical properties, notably in rotation, and the results are to be interpreted as strong evidence that the methylated derivative used had not suffered appreciable degradation during its preparation from inulin.

Hydrolysis of methylated inulin was carried out by means of aqueous methyl alcohol containing oxalic acid, these reagents being chosen after numerous preliminary experiments on the ground that under these conditions a minimum loss of material is occasioned by the formation of methoxymethylfurfural. The hydrolysis products were transformed completely into the methylfructofuranosides and distilled. Separation of the tetramethyl methylfructofuranoside from 3 : 4 : 6-trimethyl methylfructofuranoside was easily effected and the yield of the tetramethyl fructofuranose (the identity of which was controlled by its conversion into crystalline 2 : 3 : 4 : 6-tetramethyl fructofuronamide) was found to be 3·7% of the weight of inulin used. Control experiments with artificial mixtures showed that over 90% of the tetramethyl derivative could be removed from

mixtures containing a large excess of the trimethyl sugar. The whole of the hydrolysis products were examined and, in addition to 3 : 4 : 6-trimethyl fructofuranose, a small quantity (3%) of a hexamethyl difructose anhydride was obtained. The latter material had properties similar to those of the hexamethyl difructose anhydride described by Haworth and Streight (*loc. cit.*) and its structure was established as the result of its hydrolysis to 3 : 4 : 6-trimethyl fructofuranose. The difructosan is a product of depolymerisation and has been formed by recombination of fructofuranose units. Moreover the isolation of this product reveals no inconsistency with the above structure for inulin (see Bodycote, Haworth, and Woolvin, following paper).

A comparison of the present results with those briefly announced in a letter by Irvine (*Nature*, 1932, **129**, 470) on the hydrolysis of methylated inulin reveals that his yields of tetramethyl fructofuranose (1·7—2·7%) were invariably low and the references in his letter to methoxyfurfural demonstrate that the method of hydrolysis adopted in his experiments must have been inferior. We encountered none of the complications suggested in that letter.

EXPERIMENTAL.

Inulin Acetate.—Inulin (100 g.) was stirred with pyridine (1000 c.c.) at 80° for 45 mins. The mixture was cooled, stirring being continued, and to the clear solution Ac_2O (180 c.c.) was added drop by drop. After 6 hrs. (stirring) the remainder of the Ac_2O (370 c.c.) was added slowly. After a further 12 hrs. the clear solution was poured into H_2O (10 l.) and the inulin acetate (160 g.) was isolated in the usual way. It was purified by solution in hot MeOH, which on cooling deposited inulin acetate as a fine white powder, $[\alpha]_D^{20°}$ − 33° in $CHCl_3$ (*c*, 1·4). (Found: C, 49·9; H, 5·95. Calc. for $C_{12}H_{16}O_8$: C, 50·0; H, 5·6%).

Methylated Inulin.—The simultaneous de-acetylation and methylation of inulin acetate was carried out as previously described. At the end of the methylation a large amount of boiling H_2O was added to the mixture. The methylated inulin (yield, 95%) which separated at this stage was thoroughly washed with boiling H_2O, dried, and dissolved in boiling EtOH. The material which separated on cooling was dissolved in a mixture of equal vols. of acetone and $CHCl_3$. On the addition of light petroleum (b. p. 40—60°) methylated inulin was obtained, $[\alpha]_D^{20°}$ − 54° in $CHCl_3$ (*c*, 1·0) (Found: C, 53·0; H, 8·2; OMe, 45·5. Calc. for $C_9H_{16}O_5$: C, 52·9; H, 7·8; OMe, 45·6%).

Fractionation of Methylated Inulin.—Methylated inulin (20 g.) was dissolved in acetone, and to the cold solution H_2O was added with stirring. Fractions were removed after addition of 25 c.c. H_2O (7·0 g.); 50 c.c. H_2O (5·1 g.); 90 c.c. H_2O (4·1 g.); 140 c.c. H_2O (2·3 g.); 200 c.c. H_2O (1·2 g.). By this time less than 0·3 g. remained in solution. All fractions had the same solubilities, showed m. p. 140°, $[\alpha]_D^{20°}$ − 54° in $CHCl_3$, and gave on analysis OMe, 45%.

Methylated inulin (25 g.), dissolved in a mixture of acetone (35 c.c.) and $CHCl_3$ (35 c.c.), was fractionally pptd. by addition of light petroleum (b. p.

40—60°) with stirring. Six fractions (in all 24·1 g.) were examined. All had the same solubilities, m. p. 140°, and gave OMe, 44·9—45·1%. No evidence could be obtained of separation into fractions differing in any property and the methylated inulin appeared to be homogeneous.

Hydrolysis of Methylated Inulin.—Methylated inulin (40 g.) was heated at 80° with MeOH (1200 c.c.) and H_2O (400 c.c.) containing $H_2C_2O_4$ (16 g., cryst.) until the rotation was const. (18 hrs.). After neutralisation of the acid ($CaCO_3$) the filtered solution was evaporated to a syrup under diminished press. The syrup was dried by addition of EtOH and C_6H_6, followed by removal of the solvents. It was then extracted with boiling $CHCl_3$ in the presence of $MgSO_4$, giving a mobile syrup (41 g.). A solution of this syrup (16 g.) in 0·25% methyl-alc. HCl was kept for 60 hrs. at 15°. After neutralisation ($BaCO_3$) the solution was concentrated in the presence of $BaCO_3$ at 30—35° under diminished press. The product was extracted with Et_2O and on evaporation of the latter in the presence of $BaCO_3$ a non-reducing syrup (16 g.) was obtained which had no immediate action on dil. $KMnO_4$ aq. From 80 g. of methylated inulin the yield of methylated methylfructofuranosides was 80·4 g. On distillation from a bath heated at 125—130°/0·05 mm., the following fractions were obtained: A (31 g.), $n_D^{18°}$ 1·4553; B (29·2 g.), $n_D^{18°}$ 1·4555; C (6·8 g.), $n_D^{18°}$ 1·4560; D (12·1 g., still residue).

Fractional distillation of (*A*).

Fraction.	Bath temp.	B. p.	Press., mm.	Yield, g.	$n_D^{19°}$.
E	130—135°	110°	0·05	8·9	1·4545
F	130—135	110	0·05	14·0	1·4545
G	130—135	110	0·05	7·0	1·4550
Residue				0·5	

Fraction E (8·7 g.) was transferred to the Widmer flask and distilled.

Fraction.	Bath temp.	B. p.	Press., mm.	Yield, g.	$n_D^{16·5°}$.	OMe, %.
H	135—140°	up to 84°	0·03	1·45	1·4505	58·7
I	140—146	,, 93·5	0·04	2·50	1·4486	55·5
J	146—150	,, 98	0·04	2·45	1·4548	51·2
	[5·0 G. from fraction F were now added.]					
K	140—150	,, 105	0·07	3·30	1·4540	52·1
L	140—150	,, 105	0·07	1·40	1·4550	51·3

Fraction (H) contained a little ω-methoxy-5-methylfurfural, but all the other fractions were stable to $KMnO_4$. Fraction (H) was treated with excess of very dil. $KMnO_4$ aq., extracted with $CHCl_3$, and redistilled, giving 1·2 g., $n_D^{17°}$ 1·4468 (bath temp. 108°, press. 0·03 mm.). The amount of ω-methoxy-5-methylfurfural in fraction (H) could not have been more than 0·1 g.

Fractions (I), (J), and (K) were redistilled, giving a fraction (M), b. p. 84—87°/0·05 mm., $n_D^{17°}$ 1·4465.

Fraction (M) and the redistilled fraction (H) were pure tetramethyl methylfructofuranoside (2·8 g.) (Found: C, 52·5; H, 8·8; OMe, 58·8. Calc.: C, 52·8; H, 8·9; OMe, 62·0%). This total quantity of tetramethyl methylfructoside is subject to a total correction of 10% for losses (*a*) in fractional distillation (see later) and (*b*) in hydrolysis and fructoside formation, so that the quantity of the tetramethyl methylfructoside was estimated as 3·1 g., which, as the free sugar, corresponds to 3·7% of the methylated inulin used.

The tetramethyl methylfructoside ($[\alpha]_{5780}^{17°}$ + 59°) gave quantitatively on hydrolysis with 0·1*N*-HCl at 70° tetramethyl fructose, b. p. 105°/0·03 mm., $[\alpha]_{5780}^{17°}$ + 33° in H_2O (*c*, 1·0), which was characterised by its conversion into

cryst. 2 : 3 : 4 : 6-tetramethyl fructofuronamide (yield, 60%), m. p. 100° alone or when mixed with an authentic sample, $[\alpha]_{5780}^{17°}$ — 75° in H_2O (*c*, 0·7). (Found: N, 5·6; OMe, 47·0. Calc. for $C_{10}H_{19}O_6N$: N, 5·6; OMe, 49·8%). The transformation was carried out by the method of Avery, Haworth, and Hirst (J., 1927, 2313).

Examinations of the fractions of higher boiling point. All the remaining material, including (B) and (C), was distilled through the Widmer column, and yielded essentially trimethyl methylfructofuranoside: bath temp. 140—145°, b. p. 90—94°/0·025 mm., $n_D^{17°}$ 1·4555. (Found: C, 50·7; H, 8·9; OMe, 51·3. Calc.: C, 50·8; H, 8·5; OMe, 52·5%).

All these subsequent distillates were stable to $KMnO_4$ and contained no tetramethyl methylfructofuranoside.

Examination of the still residue (D). This residue (12 g.) was distilled under 0·03 mm. press. The distillation was slow and a fraction (2·5 g.; $n_D^{16°}$ 1·4643) was collected at 170—190°. This reduced Fehling's solution. The distillate (2·4 g.) was transformed into the glycoside by treatment with 0·25% methyl-alc. HCl. The glycoside distilled completely at 115°/0·03 mm., giving a mobile syrup (2·2 g.), $n_D^{16·5°}$ 1·4555.

The undistilled portion of the still residue was heated with MeOH (240 c.c.), H_2O (80 c.c.), and $H_2C_2O_4$ (3·2 g.) for 18 hrs. The acid was neutralised by pptd. $CaCO_3$, and the solution evaporated to dryness. The product, which was extracted in the usual way, was transformed into the fructofuranoside, which was dissolved in H_2O and treated with very dil. $KMnO_4$ aq. The methylfructosides were then extracted with $CHCl_3$, the solvent removed, and the mobile syrup mixed with the 2·2 g. derived from the previous series of operations (see above). This syrup (10 g.) gave the following on distillation:

Fraction.	Bath temp.	Press., mm.	Yield, g.	$n_D^{16°}$.
1	120—140°	0·03	5·5	1·4558
2	140—160	0·03	0·5	1·4570
3	160—190	0·03	0·3	1·4581
4	190—210	0·03	2·3	1·4704

Fraction (4) had $[\alpha]_{5780}^{18°}$ + 42° in $CHCl_3$ (*c*, 1·1), + 56° in H_2O (*c*, 1·99). It was a hexamethyl difructose anhydride (Found: C, 52·75; H, 7·95; OMe, 43·4. Calc. for $C_{18}H_{32}O_{10}$: C, 52·9; H, 7·8; OMe, 45·6%). On hydrolysis with 3% HCl aq. at 95° for 3 hrs. it gave in good yield 3 : 4 : 6-trimethyl fructofuranose, b. p. 125°/0·05 mm., $n_D^{18°}$ 1·4682, which was recognised as its phenylosazone, m. p. 82—83° (hydrated form), m. p. 133—134° (anhydrous form) (Found: N, 13·6. Calc.: 13·4%).

Separation of Artificial Mixtures of Tetramethyl and Trimethyl Methylfructofuranosides.—18·0 G. of authentic trimethyl methylfructofuranoside were mixed with 0·9 g. of tetramethyl methylfructofuranoside, and distilled:

Fraction.	Bath temp.	B. p.	Press. mm.	Yield, g.	$n_D^{15°}$.	OMe, %.
1A	135—140°	84—87°	0·04	0·58	1·4460	58·9
2A	135—140	84—87	0·04	0·26	1·4465	
3A	140—145	90—93	0·04	0·10	1·4495	54·2
4A	145—150	95—100	0·04	2·1	1·4550	51·6

The total recovery from 0·9 g. was at least 0·84 g. or approximately 95%.

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