LXXXIII.—*Polysaccharides. Part I. The Structure of Inulin.*

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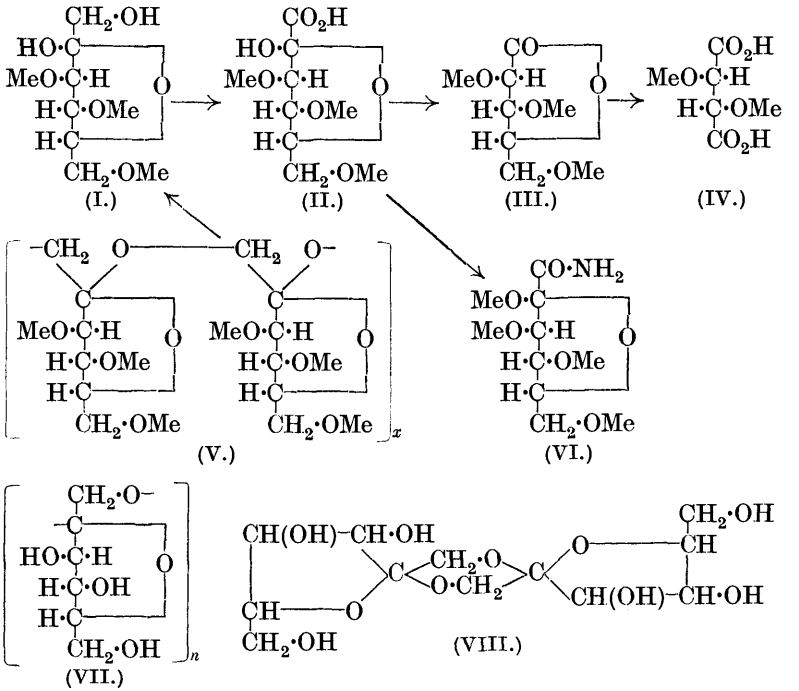
EXPERIMENTAL methods which we have found serviceable in the elucidation of the structure of the fructose component in sucrose have now been applied to the problem of the structure of inulin. From the results now communicated, a knowledge has been gained of the hydroxyl positions through which the fructose residues are linked to each other in inulin. Although this polysaccharide has been studied by previous workers and methylated to give trimethyl inulin (Irvine and Steele, J., 1920, **117**, 1474; Irvine, Steele, and Shannon, J., 1922, **121**, 1060; Karrer, *Helv. Chim. Acta*, 1921, **4**, 253) and the polysaccharide has been described as a complex of condensed γ-fructose residues, yet the characterisation of the trimethyl γ-fructose obtained from this source has not hitherto been achieved.

Inulin responded readily to methylation by means of methyl sulphate and sodium hydroxide, and, as in the case of sucrose (Haworth, J., 1915, **107**, 8), one treatment with these reagents yielded a product which was completely soluble in methyl iodide; thereafter the methylation was completed by the use of Purdie's reagents. These operations did not lead to the formation of dextrorotatory by-products as described by Irvine and his co-workers (*loc. cit.*), but gave an apparently homogeneous trimethyl inulin which separated as a white, amorphous powder. Under the present conditions it has not been found possible to avoid the use of considerable concentrations of alkali in the first methylation process, and the results now communicated are subject to the assumption that no fundamental change has occurred in the character or mode of linking of the polysaccharide due to this treatment with alkali. We have been able to detect no apparent change in the nature of inulin after subjection to this alkaline treatment. Alkali hydroxide compounds of inulin are indeed described (Pfeiffer and Tollens, *Annalen*, 1881, **210**, 285; Karrer, Staub, and Wälti, *Helv. Chim. Acta*, 1922, **5**, 129).

Hydrolysis of the trimethyl inulin with an aqueous-alcoholic solution of oxalic acid yielded a mixture of trimethyl γ-fructose (trimethyl fructo-furanose) and its ethylfructoside. The homogeneous sugar was obtained from this mixture by further hydrolysis in aqueous solution. Contrary to the observations of Irvine and his co-workers (*loc. cit.*), we have found that the trimethyl γ-fructose yields a crystalline phenylosazone, which is readily obtainable either

as a hydrated form (m. p. 80—82°) or in the anhydrous form (m. p. 137—138°). It was demonstrated that the osazone had retained the three methyl groups which were present in the methylated sugar, and therefore the positions 1 and 2 in the latter could not have been occupied by methyl groups.

Oxidation of the trimethyl γ-fructose with nitric acid gave a trimethyl lactol acid (II) which reduced Fehling's solution, and the ethyl ester of this product acquired on further methylation a fourth methyl group. This specimen of a tetramethyl lactol ester did not reduce Fehling's solution, and it was clear that the entry of the fourth methyl group had protected the reducing position. The constitution of this product was elucidated by its conversion into a crystalline amide (VI), identical with that isolated by Avery, Haworth, and Hirst (J., 1927, 2308) during the analogous transformation of the tetramethyl γ-fructose isolated from methylated sucrose.



Oxidative degradation of the trimethyl lactol acid (II) by means of acid permanganate led to the isolation of crystalline *d*-trimethyl γ-arabonolactone (III) identical with the specimen previously

obtained by oxidation of tetramethyl γ-fructose (Avery, Haworth, and Hirst) and enantiomorphously related to the specimen prepared from *l*-arabonolactone by Haworth and Nicholson (J., 1926, 1899). It has already been demonstrated that the above *d*-trimethyl γ-arabonolactone yields on further oxidation with nitric acid *l*-dimethoxysuccinic acid (IV), which has been characterised through its crystalline methylamide and by comparison of this with a specimen prepared from *l*-tartaric acid. The above lactone is thus seen to be *d*-2 : 3 : 5-trimethyl arabonolactone, and this product can only arise from a trimethyl fructose having methyl groups substituting the corresponding hydroxyl positions in the sugar, and having also a butylene oxide ring. The trimethyl fructose obtained in the above manner by cleavage of trimethyl inulin is thus seen to be 3 : 4 : 6-trimethyl fructose (I), having the oxide ring in the 2 : 5 positions (*i.e.*, adopting the nomenclature of Goodyear and Haworth, J., 1927, 3136, it is 3 : 4 : 6-trimethyl fructo-furanose). From these data, we derive the structure for trimethyl inulin (V) (p. 620).

These experimental results receive a reasonable interpretation only if it be admitted that the γ-fructose residues in inulin are mutually linked through the positions 1 and 2. We therefore derive the general structural formula (VII) for this polysaccharide. The work of Bergmann and Knehe (*Annalen*, 1926, **449**, 302) seems to indicate that triacetyl inulin has a molecular weight corresponding to a triacetyl difructose anhydride, and the latter authors have suggested that inulin may be capable of formulation on this simplified basis. If this be the case, the difructose anhydride which is said to be the unit of triacetyl inulin will receive from our experimental results the formulation (VIII). At this stage we do not, however, commit ourselves to the conclusion that inulin is an associated difructose anhydride, since the problem of the structure of polysaccharides should in our view be capable of solution on general principles of valency distribution. Inulin has probably not fewer than six of the units shown in formula (VII) (or its obvious alternative). Similarly we do not favour the hypothesis that cellulose consists of "associated" units of a di- or tri-glucose anhydride. That principal and not "residual" valency linkings are involved in the union of the component groups of glucose is supported by the evidence of *X*-ray spectrographs of polymeric compounds, and these linkings are analogous to those in, *e.g.*, cellobiose. One of us has already discussed this wider problem in a review of the subject (*Ann. Reports*, 1927, **24**, 81).

EXPERIMENTAL.

Trimethyl Inulin.—Inulin which had been specially purified and finely powdered was converted into the soluble compound with sodium hydroxide by warming in presence of alkali (compare Pfeiffer and Tollens; also Karrer, Staub, and Wälti, *loc. cit.*), and the general procedure adopted for the methylation was similar to that already described by one of us (J., 1915, **107**, 11; 1921, **119**, 198). 35 G. of inulin being dissolved as described above, there were then admitted simultaneously methyl sulphate (20 c.c.) and aqueous sodium hydroxide (50 c.c. of 35%). During this operation, the solution was vigorously stirred and the temperature, which was initially at 30°, was gradually raised to 70° and finally lowered to 30°. The same quantities of these reagents were repeatedly added under identical conditions until 200 c.c. of methyl sulphate and 500 c.c. of the alkali solution had been introduced over a period of two days. The whole of the inulin remained in solution during the course of these operations and at the end the mixture was heated at 100° for ½ hour. The excess of alkali was neutralised in presence of ice by addition of dilute sulphuric acid. To this volume of the aqueous solution half the volume of methylated spirit was added. Sodium sulphate which separated was filtered off and the neutral filtrate was evaporated under diminished pressure to a syrup, which was repeatedly extracted with chloroform. This chloroform solution and the chloroform washings from the sodium sulphate residues were combined and dried over magnesium sulphate. Distillation of the solvent yielded a pale yellow syrup which was completely soluble in methyl iodide. This product was subjected to four further methylations with Purdie's reagents, and was isolated as a white, amorphous powder after each methylation. The final product (32 g.) was washed with ether and dried at 60° in a vacuum. It melted at 138—140° to a colourless liquid which did not immediately solidify on cooling; on heating to 180° it became brown, and it did not appear to suffer much decomposition at 210°. The trimethyl inulin was practically insoluble in water or ether and showed $[\alpha]_D^{20°}$ — 50·2° (c = 1·61) in chloroform; it was very soluble in benzene and acetone and moderately easily soluble in alcohol and was devoid of action towards Fehling's solution (Found: C, 53·1; H, 7·75; OMe, 43·5. Calc.: C, 52·9; H, 7·9; OMe, 45·6%). In another preparation 32 g. of inulin were methylated and yielded 29 g. of trimethyl inulin, m. p. 138—140°, $[\alpha]_D^{20°}$ — 49·7° in chloroform (c = 2·43).

Hydrolysis. The trimethyl inulin was hydrolysed in lots of 5 g. by digesting it with a solution of 250 c.c. of 70% alcohol con-

taining 2·5 g. of oxalic acid. After heating for 10 hours, the solution attained a constant rotation and was thereupon neutralised by addition of precipitated calcium carbonate. After the mineral salts had been removed by filtration, the filtrate was evaporated under diminished pressure and the syrupy residue extracted with chloroform. The chloroform extract yielded a colourless syrup which reduced Fehling's solution actively, but it was ascertained that this syrup was a mixture of a trimethyl fructose and its ethyl-fructoside. It was therefore digested for 2 hours at 80° with aqueous hydrochloric acid (0·25%). The mineral acid was neutralised and the solution filtered and evaporated, leaving a residue which was dried and then extracted repeatedly with ether. The ethereal extracts gave on evaporation a faintly yellow syrup (4·25 g.). This distilled as a colourless liquid, b. p. about 115°/0·02 mm., having $n_D^{14^\circ}$ 1·4675; $[\alpha]_D^{20^\circ}$ + 27·7° in chloroform (c = 1·57) (Found: C, 48·45; H, 8·1; OMe, 40·2. $C_9H_{18}O_6$ requires C, 48·6; H, 8·15; OMe, 41·9%). This product was evidently a trimethyl fructose and its properties corresponded to those of a γ-sugar. It decolorised neutral permanganate instantly.

Phenylosazone. The above trimethyl γ-fructose was digested with phenylhydrazine in dilute acetic acid. There separated after heating for ½ hour at 100° an orange-red, viscid syrup, which, after having been washed with water, crystallised immediately from aqueous alcohol in a hydrated form in yellow needles, m. p. 80—82°. Yield, 84% of the theoretical (Found: C, 60·4; H, 7·4; N, 14·0; OMe, 22·2. $C_{21}H_{28}O_4N_4,H_2O$ requires C, 60·3; H, 7·2; N, 13·4; OMe, 22·2%). This hydrated osazone, when gradually heated from 50° to 100° in a high vacuum or when repeatedly crystallised from light petroleum containing a little ether, changed into the anhydrous form, m. p. 137—138°. From the solvent the *osazone* separated as a bright yellow, gelatinous mass of prisms (Found: C, 63·1; H, 7·0; N, 13·9; OMe, 22·6. $C_{21}H_{28}O_4N_4$ requires C, 63·0; H, 7·0; N, 14·0; OMe, 23·3%).

Graded Oxidation of the 3 : 4 : 6-*Trimethyl* γ-*Fructose.—Stage I. With nitric acid.* This constitution has been ascribed on the basis of the following graded oxidations.

Trimethyl γ-fructose (3 g.) was heated with 18 c.c. of nitric acid (d 1·42). The reaction became vigorous at 68° and was later maintained by heating slowly to 95°. The whole operation occupied 1½ hours. The solution was now cooled, diluted with water, and evaporated under diminished pressure during constant additions of more water. Finally, the residue was digested with ethyl alcohol, and the solvent evaporated. The residue was dissolved in ethyl alcohol containing 3% of hydrogen chloride and boiled under

reflux for 6½ hours. After having been neutralised with silver carbonate, the solution was filtered and evaporated under diminished pressure. The syrupy residue was dissolved in ether, filtered from traces of silver, and gave on evaporation a pale yellow syrup which distilled under 0·18 mm. from a bath heated at 132—140°. Yield of distillate, 2·3 g. This showed $n_D^{15°}$ 1·4529; $[\alpha]_D^{16°}$ + 27·1°; OMe, 45·7%. The product reduced Fehling's solution actively and was found by subsequent examination to consist essentially of the ester of the trimethyl lactol acid (II).

This substance was now methylated with Purdie's reagents and gave a mobile liquid which distilled under 0·1 mm. from a bath heated at 115—130°. It showed $n_D^{15°}$ 1·4453; $[\alpha]_D^{16°}$ + 2° (approx. : $c = 1·2$) in water. Solution of this methylated lactol ester in methyl alcohol and saturation with ammonia at 0° led to the formation after 2 days of the corresponding amide (VI), colourless crystals, m. p. 100—101°, from light petroleum. In admixture with a specimen of the amide prepared by Avery, Haworth, and Hirst (*loc. cit.*) from tetramethyl γ-fructose there was no depression of m. p. (Found : C, 48·2; H, 7·3; N, 5·3. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·6; N, 5·6%).

Stage II. With acid permanganate. The ethyl ester of the lactol acid, which reduced Fehling's solution and was isolated as above and as the first product of oxidation of the trimethyl γ-fructose with nitric acid, was now subjected to further oxidation as follows.

The syrupy ester (1·5 g.) was dissolved in 8·4 c.c. of *N*-sulphuric acid and 8·4 c.c. of water, and the mixture was heated at 85° for 2½ hours in order to hydrolyse the ester. There was then admitted a further quantity of *N*-sulphuric acid (8·4 c.c.) and the cooled solution was diluted to a volume of 50 c.c. The latter was then titrated slowly with a solution of barium permanganate until manganese oxides began to be precipitated. This required 9·6 c.c. of the permanganate solution (1800 c.c. = 16 g. oxygen). At this stage dilute barium hydroxide solution was admitted until the mixture was alkaline. After having been kept over-night, the mixture was treated with carbon dioxide, and the solids were filtered off and thoroughly washed with water, the washings being added to the filtrate. To the latter *N*-sulphuric acid was added in sufficient quantity to liberate the whole of the organic acid, which was present as barium salt, and the excess of mineral acid was then neutralised. The filtrate remaining from this treatment was evaporated under diminished pressure and the organic residue was extracted with ether. This appeared to leave a slight residue of a manganese salt of an organic acid, which was subsequently recovered. The ethereal extract yielded a pale yellow mobile

syrup which distilled under 0·12 mm. from a bath heated at 100—115°. This colourless distillate crystallised on being nucleated with a specimen of *d*-2 : 3 : 5-trimethyl γ-arabonolactone which had previously been prepared from tetramethyl γ-fructose by Avery, Haworth, and Hirst (*loc. cit.*). Yield, 0·4 g. This substance, recrystallised from light petroleum, had m. p. 31—32°. The identity of this product with *d*-trimethyl γ-arabonolactone was confirmed by a mixed melting-point determination and by the following analysis (Found : C, 50·4; H, 7·4; OMe, 47·7. Calc. : C, 50·5; H, 7·4; OMe, 48·9%).

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