143. *The Structure of the Fructosan from* Agave vera cruz *Mill.*

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Fractionation of the fructosan from *Agave vera cruz* Mill gave fractions containing an average of one glucose residue per molecule. Hydrolysis of the methylated fructosan afforded 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, and 1 : 3 : 4 : 6-tetra-, 3 : 4 : 6-tri-, 1 : 3 : 4-tri-, and 3 : 4-di-*O*-methyl-D-fructose in the approximate molar ratio of 1 : 8 : 10 : 5 : 7. Evidence was obtained for sucrose as a product of partial acid-hydrolysis of the fructosan. The greater part of the material possesses, therefore, a molecular structure comprising a highly branched chain of *ca.* 30 2 : 1- and 2 : 6-linked β-D-fructofuranose residues terminated by a non-reducing D-glucopyranose residue as in sucrose.

FRUCTOSANS from plants [1] are characterised by chains of 2 : 1- and 2 : 6-linked β-D-fructofuranose residues, the former mode of linkage being typical of inulin and the latter typical of the grass levans. In addition to these essentially linear polysaccharides, highly branched

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[1] Hirst, *Proc. Chem. Soc.*, 1957, 193.

fructosans containing both types of linkage have been encountered. Chemical investigations have shown that several of these fructosans contain within their molecular structure a sucrose moiety and it is probable that the polysaccharides are end-products of the transfructosylation by which the lower fructose-containing oligosaccharides are known to be synthesised from sucrose. A fructosan is the major carbohydrate constituent of the stem of *Agave vera cruz* Mill,[2] and we are grateful to Dr. M. Srinivasan for placing at our disposal a quantity of this material for structural investigation, the results of which are described in this paper.

The fructosan was fractionated by precipitation from aqueous solution with ethanol. Samples of the fractions were hydrolysed and the proportions of fructose and glucose formed were determined. Molecular-weight determinations, by the isothermal-distillation method, of the fructosan acetates indicated that the fractions contained series of polymer homologues, each fructosan molecule containing on the average one glucose residue. The negative optical rotations of the fructosan acetates ($[\alpha]_D$ −16·5° to −9°) suggested that the fructosan approximated to the inulin rather than to the levan type.[3] Periodate oxidation indicated the consumption of $n + 1$ moles of reagent per molecule of n hexose residues with the liberation of one mole of formic acid per mole of fructosan. Since no sugar residues remained unattacked by the reagent, it is possible that each fructose residue consumed one mole of periodate, whilst the glucose residue consumed two moles of reagent with release of formic acid. The largest polysaccharide fraction was then selected for more detailed study.

The fructosan was converted into its fully methylated derivative which had a number-average molecular weight of 6300 ± 500 (degree of polymerisation, 31 ± 2). Hydrolysis of the methylated polysaccharide gave a mixture of sugars from which three fractions containing tetra-, tri-, and di-*O*-methylhexose were obtained after chromatography on cellulose. The first fraction contained 1 : 3 : 4 : 6-tetra-*O*-methyl-D-fructose (27%) and 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose (2·65%), and the sugars were separated chromatographically after preferential conversion of the former sugar into its methyl glycoside.[4] The second fraction contained a mixture of 3 : 4 : 6- (33%) and 1 : 3 : 4-tri-*O*-methyl-D-fructose (16%), and separation of these two sugars was effected after conversion of the former into its *O*-*iso*propylidene derivative.[5] The third fraction contained 3 : 4-di-*O*-methyl-D-fructose. The five methylated sugars isolated on hydrolysis of the methylated fructosans were obtained in an approximate molar ratio of 8 : 1 : 10 : 5 : 7.

It follows that the fructosan from *Agave vera cruz* Mill is a highly branched polysaccharide in which both 2 : 1- and 2 : 6-linked β-D-fructofuranose residues are present. D-Glucose residues are present solely as non-reducing end groups and must therefore be an integral part of the fructosan molecule. The quantity of tetra-*O*-methyl-D-glucose isolated on hydrolysis of the methylated polysaccharide indicated that each fructosan molecule contained one such glucose residue. Further evidence for the nature of the glucose residues came from the isolation of a disaccharide, identified as sucrose by chromatography and by hydrolysis to glucose and fructose, as a product of partial acid-hydrolysis of the polysaccharide. It follows that the branched fructosan molecules are terminated by non-reducing D-glucopyranose residues linked as in sucrose. Sucrose has been similarly isolated previously from perennial ryegrass levan [6] and from inulin.[7]

These results show that this polysaccharide belongs to the same general family as other plant fructosans.[1] It most closely resembles triticin from couch grass [8] and the fructosan from the Hawaiian Ti plant,[5b] but differs from the latter polysaccharide in containing

[2] Srinivasan and Bhatia, *Biochem. J.*, 1953, **55**, 286.

[3] Schlubach and Sinh, *Annalen*, 1940, **544**, 111.

[4] R. Montgomery and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 446.

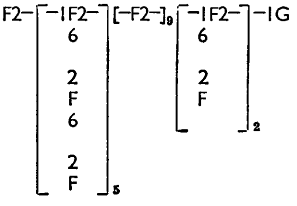
[5] (*a*) E. M. Montgomery, *ibid.*, 1934, **56**, 419; (*b*) Boggs and Smith, *ibid.*, 1956, **78**, 1880.

[6] Aspinall and Telfer, *J.*, 1955, 1106.

[7] Feingold and Avigad, *Biochim. Biophys. Acta*, 1956, **22**, 196; Holzer, Wittmann-Zinke, and Zinke, *Monatsh.*, 1957, **88**, 268.

[8] Arni and Percival, *J.*, 1951, 1822.

glucose residues as non-reducing end-groups rather than in the middle of the molecule. A unique structure cannot be advanced for the fructosan since methods are not yet available to indicate the distribution of 2 : 1- and 2 : 6-linkages in such branched polysaccharides, but the following structure is one of several possibilities. As this highly branched fructosan, like perennial ryegrass levan and inulin, contains a sucrose moiety, the recent isolation of the branched tetrasaccharide, bis-($2 \longrightarrow 1_F$, $2 \longrightarrow 6_F$)-*O*-D-fructofuranosylsucrose, from rye stems[9] is of particular interest since this is the simplest branched oligosaccharide in which both the linkages characteristic of fructosans are present.



F = D-fructofuranose and G = D-glucopyranose residues.

EXPERIMENTAL

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (B) ethyl acetate–acetic acid–water (3 : 1 : 3, upper layer); (C) butan-2-one, saturated with water; (D) light petroleum (b. p. 60—80°)–butan-2-one (1 : 1), saturated with water; (E) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3, upper layer). Paper ionophoresis was in borate buffer at pH 10.

Number-average molecular weights of acetylated and methylated polysaccharides were determined in benzene solution by the isothermal-distillation method.[10]

The fructosan was received as a white crystalline substance, $[\alpha]_D^{20}$ $-34°$ (*c* 1·6 in H_2O) (Found: ash, 0·9%). Hydrolysis of a sample gave fructose and glucose in the proportion of 20 : 1.

Fractionation of Fructosan.—Fructosan (25·7 g.) was dissolved in water (325 ml.) and fractionation was effected by gradual addition of ethanol. The precipitated gels were dried after thorough washing with acetone. Samples (100 mg.) were hydrolysed in aqueous 1% oxalic acid (10 ml.) at 75° for 1·5 hr. After neutralisation with calcium carbonate, the hydrolysates were de-ionised with Amberlite resins IR-120(H) and IR-4B(OH), and the proportions of fructose and glucose were estimated by quantitative paper chromatography[11] with solvent B. Samples were acetylated in formamide by acetic anhydride and pyridine at 0° for 3 days. The acetylated polysaccharides were isolated by pouring the reaction mixture into water and were purifed by precipitation with light petroleum from chloroform solution. The following results were obtained:

Fraction	EtOH (%) in precipitant	Wt. (g.)	$[\alpha]_D$ (H_2O)	Fructose : glucose in hydrolysate	Acetylated fructosan $[\alpha]_D$ ($CHCl_3$)	Acetylated fructosan DP_n
1	75	0·5	—	—	—	—
2	82	16·9	−38°	34 : 1	−16·5°	32
3	88	4·0	−35°	20 : 1	−14°	18
4	91	0·8	−32·5°	16 : 1	−10°	16
5	Residue not ppted.	3·1	−24°	8 : 1	−9°	15 *

* The low yield of this sample of acetylated fructosan probably resulted in the recovery of the higher-molecular part of the fraction.

Periodate Oxidation.—Fructosan samples (250 mg.) were dissolved in water (30 ml.) and 0·25M-sodium metaperiodate (20 ml.) was added. The uptake of periodate, determined by the arsenite method, was constant after 43 hr. at values of 1·03, 1·06, 1·16, and 1·36 moles of periodate consumed per hexose residue for fractions 2—5 respectively. No sugars were detected on hydrolysis of periodate-oxidised fructosan (fraction 2).

[9] Schlubach and Koehn, *Annalen*, 1958, **614**, 126.

[10] Gee, *Trans. Faraday Soc.*, 1940, **36**, 1162.

[11] Flood, Hirst, and Jones, *J.*, 1948, 1679.

Oxidation of samples with potassium metaperiodate gave the following results (expressed as the number of hexose residues per mole of formic acid liberated) (constant after 216 hr.): 34, 23, 18, and 6 for fractions 2—5 respectively.

Methylation of Fructosan.—Fructosan (fraction 2, 6·2 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide and then with methyl iodide and silver oxide to give methylated fructosan (4·8 g.), $[\alpha]_D$ $-53·5°$ (*c* 1·3 in $CHCl_3$) (Found: OMe, 45·6%; DP_n, 31).

Methylated fructosan (3·2 g.) was refluxed with methanol (120 ml.) and water (40 ml.) containing oxalic acid (2 g.) for 22 hr. {$[\alpha]_D$ $-56° \longrightarrow +30·5°$ (const.)}. The solution was concentrated to remove methanol, water (136 ml.) containing oxalic acid (1·2 g.) was added to the resulting solution, and the mixture was heated at 80° for 1·5 hr. ($[\alpha]_D$ $+34° \longrightarrow +5·5°$). The cooled solution was neutralised with calcium carbonate, filtered and concentrated to a syrup. Paper chromatograms run in solvent A and sprayed with aniline oxalate and urea oxalate for aldoses and ketoses showed tetra-*O*-methylglucose, and tetra-, tri-, and di-*O*-methylfructoses. Chromatography in solvent C showed two tri-*O*-methylfructoses. The syrupy mixture of sugars was separated on cellulose (60 × 3·5 cm.) with solvent C, to give three fractions.

Fraction 1. After careful removal of organic solvent by evaporation with water, the syrup was diluted with water to 100 ml. Chromatography showed 1 : 3 : 4 : 6-tetra-*O*-methylfructose and 2 : 3 : 4 : 6-tetra-*O*-methylglucose, and estimation of ketose colorimetrically[8] and of aldose by alkaline hypoiodite showed the two sugars to be present in the fraction to the extent of 990 mg. and 97 mg. respectively. After removal of water, the syrupy mixture of sugars was treated with methanolic 0·5% hydrogen chloride (80 ml.) at room temperature for 8 hr. to effect preferential methyl glycoside formation of the fructose derivative.[4] After neutralisation and removal of methanol the mixture was separated on cellulose (60 × 1·7 cm.) with solvent D, to give fractions 1*a* and 1*b*. Hydrolysis of fraction 1*a* with 0·2N-sulphuric acid (20 ml.) and methanol (7·5 ml.) on a boiling-water bath for 6 hr. furnished 1 : 3 : 4 : 6-tetra-*O*-methyl-D-fructose (642 mg.), $[\alpha]_D^{18}$ $+32·8°$ (*c* 1·2 in H_2O) (Found: OMe, 52·8. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%), which was characterised by conversion into methyl 3 : 4 : 6-tri-*O*-methyl-D-fructofuranosiduronamide, m. p. 98—99°, $[\alpha]_D^{18}$ $-71°$ (*c* 1·3 in H_2O). Fraction 1*b* (69 mg.) was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, m. p. and mixed m. p. 78—84°, $[\alpha]_D^{18}$ $+87°$ (equil.) (*c* 0·8 in H_2O) (Found: OMe, 52·1. Calc. for $C_{20}H_{10}O_6$: OMe, 52·5%).

Fraction 2. Chromatography of the syrup (1·657 g.) in solvent C showed 1 : 3 : 4- and 3 : 4 : 6-tri-*O*-methylfructose. In order to convert the 3 : 4 : 6-trimethyl ether into the *O*-*iso*propylidene derivative,[5] the syrup (1·32 g.) was kept in acetone (25 ml.) containing concentrated sulphuric acid (0·15 ml.) at $-20°$ for 48 hr. The solution was neutralised with sodium carbonate, filtered, and concentrated to a syrup which was separated on cellulose (60 × 3·5 cm.) with solvent C to give fractions 2*a* and 2*b*. Fraction 2*a* containing the *O*-*iso*propylidene derivative was hydrolysed with 2·25% oxalic acid (25 ml.) at 80° for 2 hr. to furnish 3 : 4 : 6-tri-*O*-methyl-D-fructose (715 mg.), $[\alpha]_D^{18}$ $+21·3°$ (*c* 2·0 in $CHCl_3$) (Found: OMe, 42·0. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). The sugar was characterised by oxidation with lead tetra-acetate (cf. Lindberg and Wickberg [12]), followed by treatment of the derived lactone with methanolic ammonia, to give 2 : 3 : 5-tri-*O*-methyl-D-arabonamide, m. p. 136—137°, $[\alpha]_D^{18}$ $-14·2°$ (*c* 2·1 in H_2O) (Found: OMe, 45·1. Calc. for $C_8H_{17}O_5N$: OMe, 44·9%). The *X*-ray powder photograph of the amide was identical with that of 2 : 3 : 5-tri-*O*-methyl-L-arabonamide. Fraction 2*b* (331 mg.) was chromatographically and ionophoretically pure and after recrystallisation from carbon tetrachloride–light petroleum (b. p. 40—60°) had m. p. and mixed m. p. (with 1 : 3 : 4-tri-*O*-methyl-D-fructose) 72—73° and $[\alpha]_D^{18}$ $-50°$ (equil.) (*c* 1·0 in H_2O) (Found: OMe, 41·5. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Fraction 3. The sugar (681 mg.), $[\alpha]_D^{18}$ $-50·3°$ (*c* 1·0 in H_2O) (Found: OMe, 29·65. Calc. for $C_8H_{16}O_6$: OMe, 29·8%), was characterised as 3 : 4-di-*O*-methyl-D-fructose by conversion into methyl 3 : 4-di-*O*-methyl-D-fructofuranoside-1 : 6-dicarboxydiamide, m. p. 191° and $[\alpha]_D^{18}$ $-68°$ (*c* 1·6 in H_2O) (Found: OMe, 37·6. Calc. for $C_9H_{16}O_6N_2$: OMe, 37·5%). The sugar was further identified by oxidation with lead tetra-acetate, followed by treatment of the derived lactone with methanolic ammonia, to give 2 : 3-di-*O*-methyl-D-arabonamide, m. p. 156—157°, $[\alpha]_D^{18}$ $-13·6°$ (*c* 1·4 in H_2O) (Found: OMe, 32·3. Calc. for $C_7H_{15}O_5N$: OMe, 32·1%). The amide gave an *X*-ray powder photograph and an infrared spectrum identical with those of 2 : 3-di-*O*-methyl-L-arabonamide.

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[12] Lindberg and Wickberg, *Acta Chem. Scand.*, 1953, **7**, 969.

Hypoiodite Oxidation of Methylated Fructoses.—Estimation, by the hypoiodite method,[13] of the " aldose " content of fractions 2 and 3 gave values of 6·4 and 3·6%. Control experiments with synthetic samples of 3 : 4 : 6-tri- and 3 : 4-di-*O*-methyl-D-fructose gave values for apparent aldose content of 6·1 and 3·5%, whereas 1 : 3 : 4 : 6-tetra- and 1 : 3 : 4-tri-*O*-methyl-D-fructose gave values of 1·4 and 1·1%. The latter values probably arise from experimental error, but the larger values for apparent aldose content of D-fructose derivatives not containing a 1-substituent may arise from ketose–aldose interconversion under the alkaline conditions employed.

Partial Acid Hydrolysis of Fructosan.—The fructosan (unfractionated; 16 g.) was heated in water (800 ml.) containing oxalic acid (4 g.) at 75° for 15 min. The solution was neutralised with calcium carbonate, filtered, and concentrated to a syrup. Chromatography in solvent E showed fructose and a series of oligosaccharides. One of the components was chromatographically indistinguishable from sucrose and gave glucose and fructose on hydrolysis.

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[13] Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289.