399. *Studies on Fructosans. Part II.* Triticin from the Rhizomes of Couch Grass* (Triticum repens *L.*).

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From the hydrolysis products of methylated triticin, tetramethyl fructofuranose (*ca.* 42%), 1 : 3 : 4- (*ca.* 11%) and 3 : 4 : 6-trimethyl fructose (*ca.* 4%), and 3 : 4-dimethyl fructose (*ca.* 39%) have been separated and identified. A highly branched structure is proposed for the polysaccharide which contains about equal numbers of fructofuranose residues linked by 2 : 1- and 2 : 6-linkages.

It appears probable that the molecule is built up from *ca.* 30 fructose residues and that there is no terminal reducing fructose residue. It is suggested that the fructofuranose aggregate is terminated by a glucose residue linked as in sucrose (see Part I, *J.*, 1950, 1297), although the alternative possibility of a closed loop cannot be entirely discounted.

Schlubach and Sinh (*Annalen*, 1940, **544**, 111) have suggested that fructosans may be divided into two main groups, namely, the inulin group in which the fructofuranose units are linked through $C_{(1)}$ and $C_{(2)}$, and the phlein group in which $C_{(2)}$ and $C_{(6)}$ linkages are fundamental. The work of Schlubach and Peitzner (*ibid.*, 1937, **530**, 120) appeared to show that the fructosan triticin did not belong to either of these classes.

Triticin occurs in the rhizomes of a common weed-type grass *Triticum repens* L. (*Agropyron repens* Beauv.). This species along with others of similar habit is commonly referred to as couch, twitch, quack-grass, etc. These grasses spread freely by means of the creeping root-stocks or rhizomes. The Greeks and Romans appear to have used couch-grass roots as a specific for bladder disorders; some use of this material as an animal feeding stuff has been reported from Scandinavia, and comparative experiments have shown it to have a high nutritive value (Wilson, *Nature*, 1945, **155**, 671).

An early attempt to extract a carbohydrate from couch grass is recorded by Stenhouse (*J.*, 1843, **2**, 139) who stated that " the grass roots certainly contain a great deal of uncrystallisable sugar which readily ferments." In 1873 Müller (*Arch. Pharm.*, **202**, 500) gave the name triticin to the gummy carbohydrate which he extracted from powdered couch-grass roots, recorded a specific rotation ($[\alpha]_D$ $-41°$ in water), and suggested that it was isomeric with sucrose. Reidemeister (*Pharm. Z. für Russl.*, 1880, **19**, 658) made a comparative study of " lævulin," sinistrin (from the bulbs of *Scilla maritima*), and triticin, and found that the last was the most readily hydrolysed by boiling water. The fructosan from *Dracæna australis* (Ekstrand and Johanson, *Ber.*, 1887, **20**, 3310; Ekstrand and Mauzelius, *Vetensk. Akad. Forhandl.*, 1889, 157) and Tanret's levosin (*Bull. Soc. chim.*, 1891, [iii], **5**, 724) isolated from the unripe grains of rye, wheat, and barley have been shown to resemble triticin in physical properties, but the only detailed structural investigation on record is that by Schlubach and Peitzner (*loc. cit.*). After painstaking purifications both of the polysaccharide itself and of the acetate, followed by deacetylation and methylation, a methyl triticin ($[\alpha]_D$ $-61°$) was obtained which was hydrolysed, and three fractions were obtained by the distillation of the benzoates in a high vacuum. In this way a tetramethyl, a trimethyl, and a dimethyl D-fructose were separated in the ratios of 3 : 1 : 3. The tetramethyl fructose was shown to be tetramethyl fructofuranose, but neither the trimethyl nor the dimethyl fructose was identified. Cryoscopic determinations of the molecular weight of triticin indicated a value of 2600—2830 corresponding to a molecule constructed from 16—18 fructose residues. Since the methylation studies appeared to show that the fructose residues were associated in groups of seven and triticin is virtually non-reducing, Schlubach and Peitzner suggested a branched cyclic formula containing 14 or 21 building units although the nature of the linkages involved was obscure.

In the present investigations couch-grass rhizomes gathered in spring gave triticin in a yield of *ca.* 12%. Several methods of extraction were attempted and a product having $[\alpha]_D$ $-48°$ (in water) (cf. $[\alpha]_D$ $-51°$, Schlubach and Peitzner, *loc. cit.*) was isolated. Hydrolysis with oxalic acid gave fructose (*ca.* 92%) which was estimated by a modification of Roe's method

* Part I, *J.*, 1950, 1297.

(*J. Biol. Chem.*, 1934, **107**, 15) communicated by Dr. D. J. Bell. By paper chromatography (Hirst and Jones, *J.*, 1949, 1659) glucose (*ca.* 5%) was also found in the hydrolysate. Methyl triticin ($[\alpha]_D$ −61°), prepared from the corresponding acetate, was fractionated and remethylated by the thallium method (OMe, 44·8%). After hydrolysis the methylated sugars were separated on a cellulose column (Hough, Jones, and Wadman, *J.*, 1949, 2511) into three main fractions. Fraction I (44%) consisted of tetramethyl fructofuranose (41·6%) and a tetramethyl aldose (2·2%). Fraction II (15%) proved to be a mixture of 3 : 4 : 6-trimethyl fructose (*ca.* 4%) and 1 : 3 : 4-trimethyl fructose (*ca.* 11%) together with a small quantity of trimethyl aldose. In one experiment with a different specimen of methylated triticin the two trimethyl fructoses were separated by use of a 27″ cellulose column, and 1 : 3 : 4-trimethyl fructose was obtained crystalline. Separation was more readily achieved, however, by condensation with acetone to give 3 : 4 : 6-trimethyl 1 : 2-*iso*propylidene fructose (Montgomery, *J. Amer. Chem. Soc.*, 1934, **56**, 419) which was separated from unchanged 1 : 3 : 4-trimethyl fructose by continuous extraction with light petroleum. From the value of the specific rotation of fraction II ($[\alpha]_D$ −33° in water) and the values for 1 : 3 : 4-trimethyl fructose ($[\alpha]_D$ −61·6°) (Bell and Palmer, *J.*, 1949, 2522) and 3 : 4 : 6-trimethyl fructose ($[\alpha]_D$ +30°) it appears that *ca.* 30% of the latter isomer was present in the mixture. From the change in specific rotation in acetone containing hydrogen chloride a slightly lower proportion (*ca.* 25%) of 3 : 4 : 6-trimethyl fructose was indicated. Fraction II on oxidation with periodate gave formaldehyde in a yield of 91% when treated under the conditions described by Bell (*J.*, 1948, 992) and Bell, Palmer, and Johns (*J.*, 1949, 1536) who found a theoretical yield of formaldehyde to be liberated from 1 : 3 : 4-trimethyl fructose and an 82% yield from the 3 : 4 : 6-trimethyl isomer. Since the only other possible trimethyl fructofuranose which would condense with acetone is the 1 : 4 : 6-trimethyl derivative (Montgomery, *loc. cit.*) and this substance cannot give formaldehyde on oxidation with periodate, the possibility that this isomer was present in fraction II can be dismissed.

Fraction III was a dimethyl hexose and made up 39% of the total hydrolysate. The specific rotation ($[\alpha]_D$ −61°) suggested that it was principally 3 : 4-dimethyl fructose although hypoiodite oxidation indicated slight contamination with a dimethyl aldohexose (1·6%). Oxidation with sodium metaperiodate yielded 1·6—2 moles of formaldehyde per $C_8H_{16}O_6$ according to the experimental conditions employed. Osazone formation gave 3 : 4-dimethyl glucosazone, principally in the form of a hydrate, although a specimen identical with authentic 3 : 4-dimethyl glucosazone was isolated on seeding with a crystal kindly supplied by Dr. D. J. Bell. Conclusive proof that fraction III was 3 : 4-dimethyl fructose was afforded by oxidation with periodic acid followed by bromine water and esterification, to give a good yield of dimethyl (−)-D-dimethoxysuccinate which was fully characterised as its crystalline amide and methylamide (Haworth and D. I. Jones, *J.*, 1927, 2349).

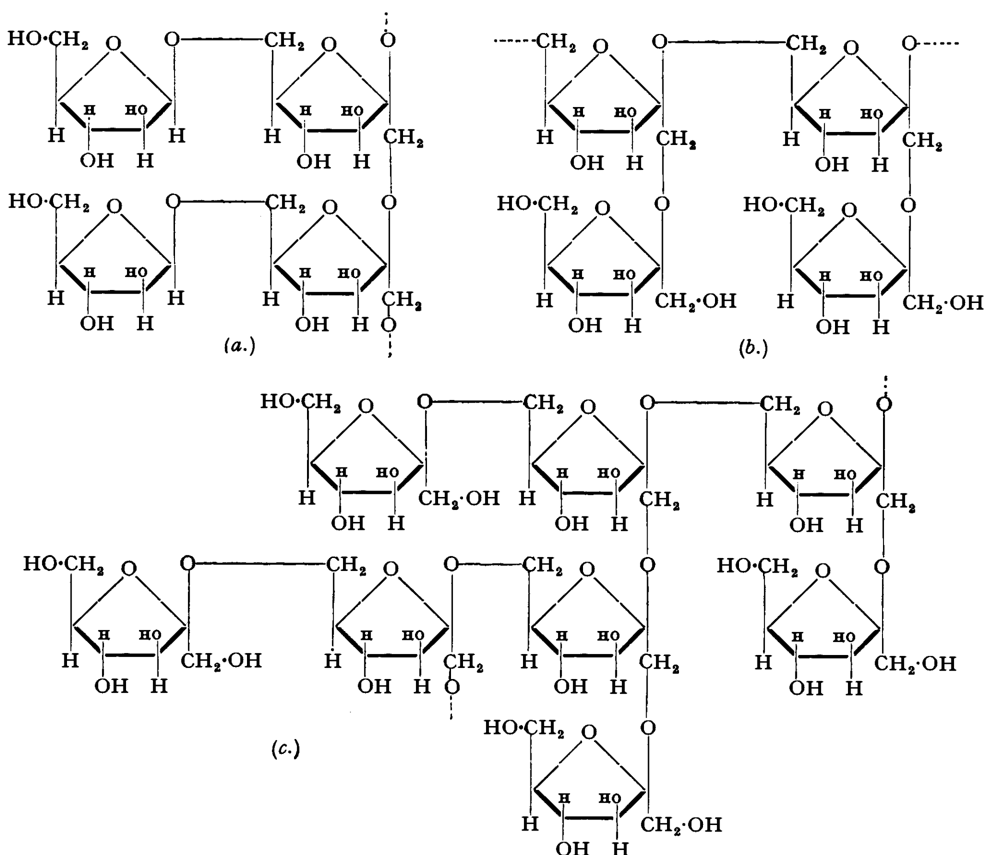
From these experiments the proportions of the various methylated fructoses produced on the hydrolysis of methylated triticin are tetramethyl fructofuranose (*ca.* 42%), 1 : 3 : 4- (*ca.* 11%) and 3 : 4 : 6-trimethyl fructose (*ca.* 4%), and 3 : 4-dimethyl fructose (*ca.* 39%). The proportions of tetra- to tri- to di-methyl fructose approximate to 3 : 1 : 3 as reported by Schlubach and Peitzner (*loc. cit.*) but the trimethyl fructose fraction is now shown to be a mixture of two known isomers rather than a single compound of unknown constitution, and the dimethyl fructose is definitely identified.

By neglecting for the time being the existence of glucose units in the molecule it is possible to indicate the main structural features of triticin. It could be depicted as built up from fructofuranose units linked either through $C_{(2)}$ and $C_{(1)}$ with branches at $C_{(6)}$ on most of these residues as (*a*) (inulin type), or through $C_{(2)}$ and $C_{(6)}$ with branches at $C_{(1)}$ as (*b*) (phlein type), or by any combination of these two possibilities such as (*c*).

The results of oxidation with sodium periodate would agree with any of these interpretations, since one equivalent is used for each $C_6H_{10}O_5$ unit, as would be expected for fructofuranose units with the adjacent hydroxyl groups on $C_{(3)}$ and $C_{(4)}$ open to attack. Triticin may therefore be represented, as far as the arrangement of the fructofuranose units is concerned, as a highly branched structure containing a random distribution of about equal numbers of 2 : 1- and 2 : 6-linked D-fructofuranose residues. That the polysaccharide is a mixture of inulin and levan (poain) is quite out of the question because of the high proportions of tetra- and di-methyl fructoses produced on hydrolysis, although the possibility that the 1 : 3 : 4-trimethyl fructose is derived from poain present as an impurity, and the 3 : 4 : 6-trimethyl fructose from a trace of inulin, cannot be completely discounted.

In common with inulin the molecular size of triticin would appear to be small in comparison with most glucosans. Schlubach and Peitzner (*loc. cit.*) estimated it to contain

ca. 18 fructose residues per molecule, and, although an accurate method for determining the molecular weight was not available to us, approximate measurements on the acetate and on the methylated derivative by the modification of Barger's method (Caesar *et al.*, *J. Amer. Chem.*

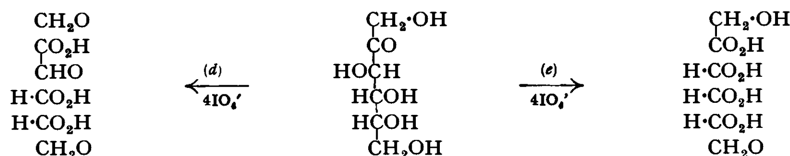


Soc., 1947, **69**, 617) previously used in this laboratory (*J.*, 1950, 3494) gave values for methylated triticin corresponding to 18—25 $C_9H_{16}O_5$ units, and for the acetate to 29 $C_{12}H_{16}O_8$ units per molecule. Since triticin is virtually non-reducing the German school favour a looped structure for this and many other fructosans. Such a possibility cannot be denied but there is another alternative. The chain could well be terminated by a glucopyranose non-reducing end-group, linked to the penultimate fructofuranose residue by a sucrose-type linkage as suggested for inulin (Hirst, McGilvray, and Percival, *J.*, 1950, Part I); Dedonder, *Compt. rend.*, 1950, **230**, 549; 997), and several lines of evidence lend support to this view.

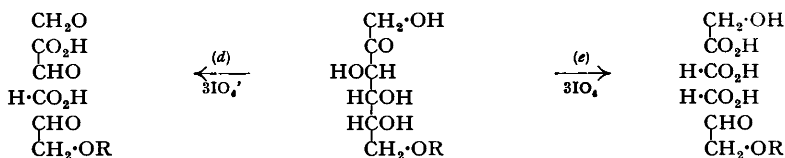
The presence of glucose among the hydrolysis products of triticin has been demonstrated beyond doubt, and the quantity determined corresponds to one glucose residue in *ca.* 21 $C_6H_{10}O_5$ units. As Reidemeister (*loc. cit.*) found, triticin was hydrolysed with ease by boiling water. An examination of the products of this autohydrolysis on the paper chromatogram showed that sucrose (R_G 0·029) was almost certainly present and it is difficult to visualise how sucrose could be produced other than by a fragmentation of the triticin molecule. Since sucrose could not be formed directly from a looped structure with a glucopyranose residue attached as a branch to one of the fructofuranose units, because the potential reducing group of the fructose residue would necessarily be joined to either $C_{(1)}$ or $C_{(6)}$ of its neighbour, the only alternative seems to be the fission of sucrose from the end of a chain. Further investigations on this autohydrolysis process are necessary, however, in view of the indications that another disaccharide (R_G 0·056) is present in the mixture of products obtained.

Oxidation with potassium periodate gave formic acid corresponding to the liberation of 1 mole per 29 hexose units and no formaldehyde could be detected. This is strong evidence

against the presence of a terminal reducing fructose group. The oxidation of fructose by periodate is complicated by the fact that it can proceed in two ways. :



although scheme (*d*) appears to take place preferentially (Khouvine and Arragon, *Bull. Soc. chim.*, 1941, **8**, 676). The hypothetical fructose reducing end-group could reasonably be expected to be joined to the main chain through either $C_{(1)}$ or $C_{(6)}$. In the former case mechanism (*d*) could not operate and we should expect the liberation of formaldehyde together with 3 mols. of formic acid. Oxidation of the 6-substituted fructose could proceed by (*d*) to give formaldehyde and 2 mols. of titratable acid or by (*e*) to give 3 mols. of titratable acid but no formaldehyde.



From the standpoint of the failure to detect formaldehyde on oxidation of triticin with periodate, the balance of evidence strongly favours the assumption that no reducing fructose end-group is present, since, in view of the observations of Khouvine and Arragon (*loc. cit.*) it is unlikely, even if the attachment were through $C_{(6)}$, that oxidation would proceed exclusively by route (*e*). Oxidation by any of the above processes would also give rise to either 3 or 2 equivalents of titratable acid. Since it was found that one equivalent of acid was released from *ca.* 29 $C_6H_{10}O_5$ units, the calculated chain length on this basis would be 58—87 units or two—three times the figure indicated by the molecular-weight measurements, so that this evidence also points to the absence of a fructose reducing group. The observed facts would fit in, however, with the assumption that the fructosan is terminated by a glucopyranose residue attached by its reducing group, for this would give rise to 1 mol. of formic acid, and no formaldehyde, on oxidation with periodate.

Further support for this view is furnished by an examination of fraction I of the hydrolysis products of methylated triticin. Examination on the filter-paper chromatogram and development with aniline oxalate gave a pink spot in the same place as the grey spot given by the interaction of the tetramethyl fructofuranose with urea oxalate. The same phenomenon was shown to occur with the hydrolysis products of methylated inulin (Part I, *loc. cit.*) and was due to the fact that tetramethyl glucopyranose and tetramethyl fructofuranose have practically identical R_G values. Unlike the corresponding fraction from the hydrolysis of methylated inulin which contained about 40% of the glucose derivative so that a partial separation by solvent extraction was effected, the overwhelming proportion of tetramethyl fructofuranose in the methylated triticin hydrolysate prevented the adoption of such a course in the present instance. An estimation of the quantity of reducing tetramethyl aldohexose was therefore carried out by hypoiodite oxidation, since this reagent has no action on tetramethyl fructofuranose (Part I, *loc. cit.*), and gave a result corresponding to the presence of 2·2% of tetramethyl glucopyranose in the total hydrolysate. This quantity is considerably less than that required (3·7%) for one glucopyranose end-group per 28 fructose units, but it is also considerably less than the total quantity of glucose known to be present in triticin. An examination of fraction II and of a small intermediate fraction revealed the presence of a trimethyl glucose (0·9%) chromatographically identical with 2 : 4 : 6-trimethyl glucose, and a dimethyl glucose (1·6%) was estimated to be present in fraction III. These figures are based entirely on the assumption that partly methylated fructoses are completely resistant to alkaline hypoiodite. It is by no means certain that these partly methylated glucoses have any structural significance for it is likely that they arise, in part, from incomplete methylation which might explain the apparently low yield of

tetramethyl glucose. If the figures for the methylated glucoses are taken as a whole, however, the possibility that the glucose produced by the hydrolysis of triticin is derived from an associated glucosan such as starch is negatived because of the high proportion of tetramethyl glucose to trimethyl glucose, even if the dimethyl glucose is included in the latter figure. Before the hypothesis that triticin contains a sucrose-type end-group can be fully substantiated, however, it will be necessary to develop methods for the separation of relatively small quantities of methylated glucoses from the corresponding fructofuranose derivatives.

EXPERIMENTAL.

The Free Sugar Content.—Couch-grass rhizomes (*Agropyron repens*) collected at New Graden, Roxburghshire (April, 1948), were cleaned and milled in a " Christy–Norris " hammer mill (No. 18 mesh screen). The milled roots (800 g.) were continuously extracted with 80% aqueous alcohol (3 l.) for 14 hours in a copper Soxhlet extractor. The yellow extract was concentrated under reduced pressure, alcohol being removed as an alcohol–water azeotrope, and the aqueous solution clarified by filtration through " Filter Cel " and evaporated under reduced pressure, to yield a dark, viscous syrup (103 g.).

Paper chromatography indicated the presence of glucose, fructose, and a disaccharide (?). Estimation of the simple sugars by the method of Flood, Hirst, and Jones (*J.*, 1948, 1679) gave fructose 17·4% and glucose 3·4% (mean of 2 determinations). The disaccharide was extracted from the paper, hydrolysed by heating it with N-sulphuric acid (10 c.c.) for 4 hours, neutralised with barium carbonate and concentrated to a syrup. Examination on the paper chromatogram showed glucose (mainly), fructose, and xylose.

Paper chromatograms run in an acidic solvent [acetic acid (10%)–*n*-butanol (40%)–water (50%)] and developed with *o*-phenetidine–trichloroacetic acid showed no evidence for the presence of uronic acid. The naphtharesorcinol test for uronic acid also gave a negative reaction.

The residue from the above alcoholic extraction (660 g.) was available for the extraction of the fructosan from the milled roots.

Extraction of the Fructosan.—Extracted milled couch-grass roots (10 g.) were shaken with cold water (100 c.c.) in the presence of barium carbonate and toluene for 4 hours. The aqueous extract was filtered through cotton wool, and protein removed by the addition of a saturated solution of basic lead acetate in water at 60° until precipitation appeared complete. After filtration, lead ions were removed as sulphide, hydrogen sulphide removed by aeration, the aqueous solution concentrated (40°/15 mm.), and the fructosan precipitated by pouring of the concentrated solution (20 c.c.) into alcohol (600 c.c.) dropwise with constant stirring. The white fibrous solid was reprecipitated twice from water with alcohol, washed with alcohol and ether, and dried in a vacuum-desiccator. The fine white hygroscopic powder so obtained (1·48 g.) had $[\alpha]_D^{16}$ −43° (*c*, 1 in water) [Found: ash (as sulphate), 3·5; protein, 2·0 (from Kjeldahl-N); ethanol, 1·9%] (sample A).

Other methods of removal of protein were then studied, the technique otherwise being the same as that described above.

(i) The aqueous extract was shaken with chloroform (0·25 vol.) and *n*-butanol (0·1 vol.) (Sevag *et al.*, *J. Biol. Chem.*, 1938, **124**, 425), protein being removed as a white, stable gel. After 10 repetitions of the process, protein removal appeared complete. The fine, white, hygroscopic powder, after 2 reprecipitations, had $[\alpha]_D^{16}$ −44° (*c*, 1·0 in water) [Found: ash (as sulphate), 5·0; protein, 1·6; ethanol, 1·5%] (sample B).

(ii) Addition of neutral lead acetate solution at 40° till precipitation appeared complete, followed by removal of lead ions as sulphide and removal of last traces of protein with chloroform–butanol, yielded, after 2 reprecipitations, a fine white powder which had $[\alpha]_D^{16}$ −33° in water [Found: ash, 3·5% (as sulphate)] (sample C).

(iii) Variations on the method described by Fujita and Iwatake (*Biochem. Z.*, 1931, **242**, 43) for the removal of protein with cadmium hydroxide were studied.

(*a*) To a solution of cadmium sulphate ($CdSO_4,8H_2O$; 1 g.) in water was added N-sodium hydroxide (7·8 c.c.), and the suspension of cadmium hydroxide added dropwise to the boiling aqueous extract in the presence of barium carbonate. Cadmium was removed as sulphide and the last traces of protein with chloroform–butanol. The fine, white powder had $[\alpha]_D^{15}$ −28° (in water) (sample D).

(*b*) Addition of a cadmium hydroxide suspension, as above, followed by removal of cadmium ions by elution through " Zeocarb-Deacidite " ion-exchange columns and treatment with chloroform–butanol, yielded a fine white powder with $[\alpha]_D^{15}$ −48° (in water) (sample E).

(*c*) To the boiling aqueous extract (barium carbonate present) was added cadmium sulphate (1 g.) and, after 1 minute, N-sodium hydroxide (5 c.c.), and cadmium was removed as sulphide. The product had $[\alpha]_D^{16}$ −36° (in water) (sample F).

(*d*) By using the technique outlined in (*c*) followed by removal of ionic material by elution through ion-exchange columns, a fine white powder with $[\alpha]_D^{18}$ −44° (in water) was obtained (sample G).

(iv) A specimen of impure polysaccharide (3·5 g.; $[\alpha]_D^{16}$ −29° in water) was dissolved in dry pyridine (40 c.c.) according to Pacsu and Mullen's method (*J. Amer. Chem. Soc.*, 1941, **63**, 1487). Acetic anhydride (40 c.c.) was added to the solution with stirring and the whole shaken for 20 hours. Acetylated triticin was precipitated with water (1 l.) and washed thoroughly with water, and the white powder dried in a vacuum-desiccator over calcium chloride. After reprecipitation from chloroform with light petroleum (b.p. 60—80°) a fine white powder (2·2 g.; Ac, 42·0%) was obtained with $[\alpha]_D^{17}$ −11° (*c*, 2·0 in chloroform).

The acetate was dissolved in dry chloroform (15 c.c.), cooled in a freezing mixture, and deacetylated by the addition of a solution of sodium (0·1 g.) in absolute methanol (5 c.c.). The semi-solid mixture was shaken for 5 hours, ice-water (5 c.c.), acetic acid (2 c.c.; 10%), and water (13 c.c.) were added and the deacetylated product was precipitated from the concentrated aqueous layer with alcohol. This yielded a fine, white, hygroscopic powder (0·9 g.), $[\alpha]_D^{17}$ −44° (in water) [Found: ash (as sulphate), 4·3%] (sample H).

All the samples of purified triticin described above reduced Fehling's solution slightly, after boiling gently for 15 minutes, but a similar effect was also observed with pure sucrose.

Hydrolysis of Triticin.—The purified triticin (E) was hydrolysed by heating it with oxalic acid solution (10 c.c.; 1%) at 80°, hydrolysis being complete in 10 minutes ($[\alpha]_D^{17}$ −48° ⟶ −84°, constant). After neutralisation with calcium carbonate, ions were removed by shaking with "Amberlite" ion-exchange resins [IR–100(H) and IR–4B(OH)]. Paper chromatograms run in benzene–butanol–pyridine–water (1 : 5 : 3 : 3) indicated the presence of fructose and glucose.

The fructose produced on hydrolysis was estimated directly by a colorimetric method. To 5 c.c. of a solution of "acid reagent" (130 g. of glycerol B.P., 100 c.c. of AnalaR concentrated hydrochloric acid containing 45 mg. of copper sulphate pentahydrate per l., and 50 c.c. of water) were added a solution of resorcinol in water (0·45%) (1 c.c.) and a standard fructose solution (2 c.c.), and the tube was fitted with a close-fitting glass bulb at the mouth. After thorough mixing, the contents of the tube were heated in a boiling water-bath for exactly 12 minutes, cooled at once in running water, and then read against a blank carried through the process simultaneously, the Spekker absorptiometer (1-cm. cells and filter I.S. violet 601) being used. A standard curve for fructose was constructed over the range 0·02—0·20 mg./c.c., a linear relation being found between log I/I_0 and concentration. A standard solution of pure polysaccharide ($[\alpha]_D^{16}$ −48°) containing 0·1 mg./c.c. was made, and the fructosan solution treated according to the method outlined above. From the standard graph, the fructose produced on hydrolysis was estimated as 91·8% (mean of 2 determinations).

The glucose liberated on hydrolysis of purified triticin was estimated as 4·8% after separation on the chromatogram, by oxidation with sodium metaperiodate as described by Hirst and Jones (*loc. cit.*).

Autohydrolysis of Triticin.—Purified triticin (sample B) was dissolved in water (10 c.c.) and heated on the boiling water-bath to constant rotation: $[\alpha]_D^{16}$ −44° (zero) ⟶ −46° (3 hours) ⟶ 51° (12 hours) ⟶ −54° (16 hours) ⟶ −75° (19 hours) ⟶ −81° (24 hours), constant.

The aqueous solution was concentrated to a syrup under reduced pressure, and duplicate paper chromatograms run in ethyl acetate–acetic acid–water (3 : 1 : 3). One, sprayed with naphtharesorcinol reagent, as described by Partridge (*Biochem. J.*, 1948, **42**, 238), showed the presence of fructose, a fructose derivative (R_G = 0·056) and 2 spots travelling at approximately the same rate as sucrose and turanose. The other, sprayed with ammoniacal silver nitrate, showed the presence of fructose, glucose, and a reducing spot (R_G = 0·056).

Other samples of triticin gave the same results on autohydrolysis.

Acetylation of Triticin.—Triticin (34 g.) was dissolved in dry pyridine (400 c.c.), according to Pacsu and Mullen, and acetic anhydride (400 c.c.) added with stirring. After 48 hours' shaking, the acetate was precipitated with water, washed thoroughly with water, and dried in a vacuum-desiccator over calcium chloride and finally in a vacuum oven at 40°/30 mm. over concentrated sulphuric acid. The buff-coloured solid (42 g.) was reprecipitated twice from chloroform solution with light petroleum (b. p. 60—80°), the resulting product (37·5 g.; Ac, 44·8%) having $[\alpha]_D^{15}$ −11° (*c*, 3·0 in chloroform). Fractionation of the acetate (32·7 g.) from chloroform (350 c.c.) with light petroleum (b. p. 60—80°) gave (*a*) 8·3 g. {Ac, 41·3%, $[\alpha]_D^{17}$ −9° (*c*, 3·0 in chloroform)}; (*b*) 11·5 g. {Ac, 41·9%, $[\alpha]_D^{17}$ −9° (*c*, 3·0 in chloroform)}; (*c*) 5·0 g. {Ac, 42·2%, $[\alpha]_D^{17}$ −9° (*c*, 3·0 in chloroform)}; (*d*) 6·6 g. {Ac, 42·9%, $[\alpha]_D^{17}$ −7° (*c*, 1·5 in chloroform)}; and, by evaporation, (*e*) 0·6 g. {Ac, 45·6%, $[\alpha]_D^{17}$ +2·5° (*c*, 1·5 in chloroform)}.

Viscosity measurements. The viscosities of the various fractions (1% solution in chloroform) were determined in a closed Ostwald viscometer at 20°:

Fraction.	*c*.*	Average time of flow (secs.), solvent.	solution.	$\eta_{sp.}$	$\eta_{sp.}/c$.
a	0·0371	65·4	69·2	0·058	1·567
b	0·0395	,,	69·7	0·065	1·647
c	0·0379	,,	68·5	0·047	1·242
d	0·0400	,,	67·5	0·032	0·800
e	0·0570	,,	68·7	0·050	0·877

* *c* = concn., in g.-mol. of repeating unit per litre.

Methylation of Triticin.—Fractions I and II of the fractionated acetate (19 g.) were dissolved in acetone (500 c.c.), and methyl sulphate (210 c.c.) and sodium hydroxide solution (560 c.c.; 30%) were added simultaneously with efficient stirring, the reaction being carried out at 40° and in an atmosphere of nitrogen. The acetone was then removed under diminished pressure and the temperature slowly raised to 75°, whereupon the partly methylated material was obtained as a brown, hard, solid mass. The product was methylated twice more by this procedure and was then washed free from sulphate with boiling water (2 l.). The crude pale yellow solid (11·9 g.) was then methylated with methyl iodide (85 c.c.) and silver oxide (60 g.), and the product precipitated from chloroform with light petroleum (b. p. 60—80°). This yielded a fine white powder (9·7 g.; OMe, 43·3%) which had $[\alpha]_D^{18}$ −61° (*c*, 1·0 in chloroform). Fractionation from chloroform (70 c.c.) with light petroleum (b. p. 60—80°) gave (*a*) 5·2 g. {OMe, 41·4%, $[\alpha]_D^{19}$ −60° (*c*, 1·0 in chloroform)}; (*b*) 2·44 g. {OMe, 41·0%; $[\alpha]_D^{19}$ −60° (*c*, 1·0 in chloroform)}; and, by evaporation, (*c*) 1·06 g. {OMe, 40·5%; $[\alpha]_D^{19}$ −55° (*c*, 1·0 in chloroform)}.

Viscosity measurements. These were determined in *m*-cresol solution at 20°, by means of an Ostwald viscometer.

		Average time of flow (secs.),			
Fraction.	*c*.	solvent.	solution.	$\eta_{sp.}$	$\eta_{sp.}/c$.
a	0·0163	442·4	449·1	0·015	0·920
b	0·0184	,,	445·3	0·007	0·380
c	0·0156	,,	446·2	0·008	0·512

A portion (2·05 g.) of fraction (*a*) was methylated with methyl iodide (100 c.c.) and thallous ethoxide (2·5 g.) to yield a white solid (1·92 g.; OMe, 43·4%). This was combined with a sample from a trial methylation (OMe, 43·2%), and the total (2·70 g.) again methylated with thallous ethoxide–methyl iodide to yield a fine white powder (2·56 g.; OMe, 44·6%) [Found, ash (as sulphate), 0·5%].

Hydrolysis of Methylated Triticin and Separation of Methylated Fructoses.—The methylated polysaccharide (2·46 g.) was dissolved in methanol (75 c.c.) and water (25 c.c.) containing oxalic acid (1 g.) and heated at 80° for 24 hours. Methanol was removed as a methanol–water azeotrope until finally a purely aqueous solution (1% with respect to oxalic acid) was obtained, and the solution heated at 80° for 5 hours. After neutralisation with calcium carbonate, the solution was evaporated to a syrup at 40°/15 mm., the syrup extracted with boiling chloroform, and the extract dried (Na_2SO_4) and evaporated to yield a mobile syrup (2·72 g.) which was dried in a vacuum-desiccator over phosphoric oxide.

Fractionation on the Cellulose Column.—The hydrolysate (2·70 g.) was separated into its component sugars by elution through a column of powdered cellulose (15″ × 1¼″ × 1¼″) with 50% light petroleum (b. p. 100—120°)–50% *n*-butanol saturated with water. Every tenth tube was evaporated and spotted on the chromatogram. Duplicate paper chromatograms were run, one being sprayed with urea oxalate to detect methylated fructoses, and the other with aniline oxalate to detect methylated aldoses.

The tetramethyl fraction (I), found in tubes 51—110, was estimated colorimetrically, by a process similar to that described earlier for the determination of fructose. A standard curve was constructed for chromatographically pure tetramethyl fructofuranose over a range 0·05—0·25 mg./c.c. in saturated benzoic acid solution in water, a linear relationship being found between log I/I_0 and concentration. The runs were repeated upon different standard solutions of tetraethyl fructofuranose, whereupon excellent agreement was obtained. Somewhat lower readings were obtained with a supposedly pure specimen but examination on the chromatogram showed trimethyl fructose to be present as an impurity.

Concn. (mg./c.c.)	0·25	0·20	0·175	0·150	0·125	0·10	0·075	0·050
Spekker reading (log I/I_0), chromatographically pure	0·642	0·523	0·440	0·380	0·323	0·256	0·191	0·116

In order to investigate a quantitiative method for the partitioning of tetramethyl fructofuranose from butanol–light petroleum–acetone into a saturated benzoic acid solution in water, a control experiment was performed. A solution of chromatographically pure tetramethyl fructofuranose in *n*-butanol (50%)–light petroleum (50%) saturated with water was made. The solvent was removed as an azeotrope at 40°/15 mm., a saturated solution of benzoic acid in water being added from a dropping funnel. The aqueous solution was filtered into a 1-l. graduated flask, then diluted to the mark, and the tetramethyl fructofuranose estimated colorimetrically on 2-c.c. aliquot portions. This gave an estimated recovery of 102·2%.

Fraction I from the cellulose column was then diluted to 1 l., and two 15-c.c. samples were withdrawn and submitted individually to the above colorimetric estimation. The tetramethyl fructofuranose in fraction I was estimated as 1·06 g. (mean of 2 determinations).

Fraction I was then evaporated to a syrup (40°/15 mm.), extracted with water, and filtered through "Filter Cel." After removal of the solvent by evaporation, a mobile syrup (1·098 g.) was obtained which was dried in a vacuum-desiccator over phosphoric oxide. The syrup had $[\alpha]_D^{15}$ +34° (*c*, 1·0 in water), was chromatographically pure, and gave a slight pink colour with aniline oxalate. The proportion of tetramethyl glucopyranose was estimated as 5·1% by oxidation with alkaline hypoiodite as described by Hirst, McGilvray, and Percival (*J.*, 1950, 1297). It was concluded that fraction I contained 0·056 g. of tetramethyl glucopyranose and 1·06 g. of tetramethyl fructofuranose.

The trimethyl fraction (II), found in tubes 184—451 from the cellulose column, was studied gravimetrically. This yielded a viscous syrup (0·381 g.) which was dried in a vacuum-desiccator over phosphoric oxide. The syrup (OMe, 38%; n_D^{17} 1·4688) had $[\alpha]_D^{16}$ −28° ⟶ −33·5° (*c*, 1·0 in water) and the aldose content was estimated as 4·0% by oxidation with alkaline hypoiodite, so that fraction II contained 0·366 g. of trimethyl fructose and 0·015 g. of trimethyl glucose. The dimethyl fraction (III), found in tubes 581—1020, yielded a viscous syrup (1·047 g.) which was dried in a vacuum-desiccator over phosphoric oxide. This syrup (OMe, 27·4%; n_D^{18} 1·4782) had $[\alpha]_D^{25}$ −61° (*c*, 1·0 in water). The aldose content was estimated as 3·9% by alkaline hypoiodite oxidation. Fraction III, therefore, contained 0·041 g. of dimethyl glucose and 1·006 g. of dimethyl fructose.

Intermediate III (tubes 452—580) on evaporation yielded a syrupy residue (17 mg.). Examination on the paper chromatogram showed the presence of a pink spot (aniline oxalate) travelling at the same rate as 2 : 4 : 6-trimethyl glucose. The aldose content was estimated as 49%, corresponding to 8·3 mg. of trimethyl glucose. This, combined with the aldose content of fraction II, gives a total estimate of 23·3 mg. of trimethyl glucose.

Identification of Fraction I.—The tetramethyl fructose was identified as tetramethyl fructofuranose by conversion into tetramethyl fructofuronamide, according to Avery, Haworth, and Hirst (*J.*, 1927, 2308). After three crystallisations from ether, long fine needles were obtained, having m. p. 98—100°,

not depressed on admixture with an authentic specimen, $[\alpha]_D^{16}$ −76° (*c*, 1·0 in water) [Found : C, 48·2; H, 7·7; N, 5·8; OMe, 46·6. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·6; N, 5·6; OMe, 49·8%].

Identification of Fraction II.—Treatment with periodate as described by Bell (*J.*, 1948, 992) gave 0·91 mole of formaldehyde per mole of trimethyl sugar.

Fraction II (0·202 g.) on treatment with a freshly prepared solution (0·5%) of hydrogen chloride in anhydrous acetone underwent the following change in rotation : $[\alpha]_D^{15}$ +4° (zero); +12° (5 mins.); +15° (25 mins., constant). The solution was poured into a saturated solution of sodium hydrogen carbonate (50 c.c.) and extracted continuously with purified light petroleum (b. p. 40°) in a liquid extractor until no further material was extracted. The extract was evaporated (reduced pressure), and dried in a vacuum-desiccator overnight to yield a colourless mobile syrup (42 mg.) which distilled in a high vacuum (bath-temp., 85°; pressure, 0·04 mm.). The distillate had $[\alpha]_D^{16}$ +46° (*c*, 1·0 in water) and gave a strong positive test for combined acetone (Found : OMe, 35·0%; n_D^{22} 1·4480).

After extraction with light petroleum the aqueous solution was concentrated to a syrup at 40°/15 mm. and extracted with chloroform, and the extract dried (Na_2SO_4) and evaporated to yield a viscous syrup (99 mg.) which was dried in a vacuum-desiccator over phosphoric oxide. The syrup (OMe, 38%; n_D^{20} 1·4640) had $[\alpha]_D^{16}$ −53° (*c*, 1·4 in water), was chromatographically pure, and rapidly crystallised when seeded with a crystal of 1 : 3 : 4-trimethyl fructose.

Authentic 3 : 4 : 6-trimethyl fructose, on treatment with a solution (0·5%) of hydrogen chloride in dry acetone, underwent the following change in rotation : $[\alpha]_D^{17}$ +30° (zero); +70° (5 mins.); +75° (25 mins., constant). The solution was poured into sodium hydrogen carbonate solution and extracted with chloroform, and the chloroform extract dried (Na_2SO_4) and evaporated to a syrup which distilled in a high vacuum (bath-temp., 65°; pressure 0·005 mm.). The distillate (n_D^{19} 1·4446) had $[\alpha]_D^{16}$ +64° (*c*, 0·4 in acetone) and $[\alpha]_D^{16}$ +71° (*c*, 1·2 in water).

Further proof that the trimethyl fraction was a mixture of 3 : 4 : 6- and 1 : 3 : 4-trimethyl fructose was obtained by hydrolysis of the remainder of fraction (*a*) of methylated triticin and elution through a long column of powdered cellulose (27″ × 1⅞″ × 1⅞″) with 50% *n*-butanol–50% light petroleum saturated with water. Under these conditions, the two trimethyl fructoses separated.

Trimethyl X, a viscous pale yellow syrup, travelled at the same rate on the chromatogram as authentic 3 : 4 : 6-trimethyl fructose. The syrup (OMe, 38%; n_D^{18} 1·4650) had $[\alpha]_D^{16}$ +32° (*c*, 1·5 in water) and underwent the following change in rotation in a solution of hydrogen chloride in dry acetone : $[\alpha]_D^{16}$ +31° (zero); +69° (5 mins.); +75° (25 mins., constant). The product distilled in a high vacuum (bath-temp., 85°; pressure, 0·02 mm.) as a mobile syrup (n_D^{13} 1·4510) which had $[\alpha]_D^{13}$ +64° (*c*, 1·0 in water) and which liberated acetone on acid hydrolysis.

Trimethyl Y, a viscous syrup, travelled more slowly than authentic 3 : 4 : 6-trimethyl fructose on the chromatogram. The syrup (OMe, 38%; n_D^{20} 1·4638) had $[\alpha]_D^{16}$ −51·5° (*c*, 1·0 in water). When seeded with authentic 1 : 3 : 4-trimethyl fructose, the syrup rapidly crystallised. After draining on porous tile, the crystalline material was triturated with light petroleum (b. p. 40—60°) and crystallised from light petroleum–carbon tetrachloride. The long needles, m. p. 72—73°, showed no depression of m. p. on admixture with an authentic crystal of 1 : 3 : 4-trimethyl fructose.

Identification of Fraction III.—Treatment with periodate as described by Reeves (*J. Amer. Chem. Soc.*, 1941, **63**, 1476) gave 2·0 moles of formaldehyde per mole of dimethyl sugar. Oxidation according to Bell (*J.*, 1948, 992) gave 1·64 moles of formaldehyde per mole of dimethyl sugar.

Osazone formation. To the dimethyl fructose syrup (0·18 g.) was added a solution of phenylhydrazine hydrochloride (0·7 g.) and crystalline sodium acetate (1·4 g.) in water (5 c.c.). A little sodium hydrogen sulphite was added and the mixture heated on a water-bath at 80° for 30 minutes. On cooling, a red oil separated which, on trituration with water, rapidly hardened to yield a yellow amorphous solid (0·036 g.), m. p. 74—78°. By heating of the aqueous solution at 100° for a further 4 hours, a pale yellow, amorphous solid separated (0·045 g.), m. p. 78—79° (Found : C, 60·3; H, 7·1; N, 13·9; OMe, 14·9. Calc. for $C_{20}H_{26}O_4N_4$: C, 62·2; H, 6·7; N, 14·5; OMe, 16·0. Calc. for $C_{20}H_{26}O_4N_4,H_2O$: C, 59·4; H, 6·9; N, 13·9; OMe, 15·3%).

A small sample of the material crystallised as long, fine, yellow needles, m. p. 124—126°, from ether–light petroleum (b. p. 60—80°) on being seeded with authentic 3 : 4-dimethyl glucosazone kindly provided by Dr. D. J. Bell. The m. p. of the crystalline material (Found : C, 62·5; H, 6·9. Calc. for $C_{20}H_{26}O_4N_4$: C, 62·2; H, 6·7%) was not depressed on admixture with authentic 3 : 4-dimethyl glucosazone.

Oxidation with periodic acid. To the dimethyl fructose syrup (0·82 g.) was added periodic acid (25 c.c.; 1·15M.), and the solution set aside at 15° for 36 hours. After aeration of the solution for 24 hours, barium chloride (2·3 g.) and excess of barium carbonate were added and the solution was filtered. Bromine (4 c.c.) and barium carbonate (2 g.) were added and the solution was kept at room temperature for 36 hours. Bromine was removed by aeration, the solution was neutralised with silver carbonate and filtered, and silver ions were removed as sulphide. The solution was evaporated to dryness (40°/15 mm.), the last traces of water being removed by the addition of methanol and, finally, anhydrous methanol. The residue was boiled overnight with methanolic hydrogen chloride (60 c.c.; 4%), and the solution neutralised with silver carbonate, filtered and evaporated to a syrup. The syrup was purified by extraction with chloroform and finally by distillation in a high vacuum (bath-temp., 105°; pressure, 0·2 mm.). The colourless, mobile distillate (0·55 g.) had n_D^{18} 1·4325 and $[\alpha]_D^{16}$ −77·5° (*c*, 1·7 in methanol).

A portion of the distilled dimethyl (−)-D-dimethoxysuccinate (0·167 g.) was dissolved in methanolic ammonia (2 c.c.) and set aside at 0° for 3 days. Crystals of (−)-D-dimethoxysuccinamide (0·14 g.) separated, having m. p. 278°, $[\alpha]_D^{16}$ −92° (*c*, 1·0 in water) (Found : C, 40·9; H, 6·8; N, 16·2; OMe, 34·2. Calc. for $C_6H_{12}O_4N_2$: C, 40·9; H, 6·8; N, 15·9; OMe, 35·2%).

The remainder of the distilled dimethyl (−)-D-dimethoxysuccinate (0·343 g.) was dissolved in methanolic methylamine (2 c.c.), and the solution kept at 0° for 3 days. Crystals of (−)-D-dimethoxysuccinobismethylamide (0·123 g.) separated, m. p. 207—208°. By evaporation of the filtrate, a further crop of crystalline material (0·218 g.) was obtained. On crystallisation from ethyl acetate the material separated as long needles, m. p. 208—209°, unchanged on admixture with an authentic specimen. This material had $[\alpha]_D^{16}$ −134° (*c*, 1·2 in water) (Found: C, 47·3; H, 7·85; N, 13·7; OMe, 29·5. Calc. for $C_8H_{16}O_4N_2$: C, 47·0; H, 7·8; N, 13·7; OMe, 30·4%).

The Molecular Weights of Acetylated and Methylated Triticin.—These were determined by the micromolecular-weight method of Barger (*J.*, 1904, 285), as modified for high polymers by Caesar, Gruenhut, and Cushing (*J. Amer. Chem. Soc.*, 1947, **69**, 617). Droplets of a 5·03% solution of acetylated triticin [fraction (*a*)] in chloroform were compared in capillary tubes with solutions of sucrose octa-acetate (4—8 × 10^{-3}M.), a travelling microscope accurate to 0·02 mm. being used. The isopiestic condition was found to lie at an approximate concentration of 6 × 10^{-3}M., whence the molecular weight of acetylated triticin (*a*) appears to be of the order 8380, corresponding to a chain length of 29 $C_{12}H_{16}O_8$ units.

By using a 1·13% solution of methylated triticin [fraction (*b*)] in chloroform against solutions of sucrose octa-acetate (2—9 × 10^{-3}M.), the equilibrium point was found to lie between 2 × 10^{-3} and 3 × 10^{-3}M-sucrose octa-acetate, from which the molecular weight of methylated triticin (*b*) appears to lie between 3750 and 5250, corresponding to a chain length of 18—25 $C_9H_{16}O_5$ units.

The Oxidation of Triticin with Periodate.—(i) *Uptake of periodate.* To purified triticin (0·2813 g.; sample E) in water (35 c.c.) sodium metaperiodate solution (15 c.c.; 0·3M.) was added and the solution set aside in the dark at room temperature. The amount of periodate remaining in comparison with a blank was determined by the addition of excess of 0·1N-sodium arsenite solution and potassium iodide, and titration with 0·1N-iodine [Found: 0·75 hour, 0·88; 1·5 hours, 0·92; 3·0 hours, 0·96; 20 hours, 1·00 moles per 8 $C_6H_{10}O_5$ (constant)].

(ii) *Determination of formic acid liberated.* To triticin (0·2885 g.; sample E) in water (35 c.c.), potassium chloride (1 g.) and sodium metaperiodate solution (15 c.c.; 0·25M.) were added, and the solution was shaken in the dark at room temperature. Samples (5 c.c.) were withdrawn at intervals, periodate was destroyed with ethylene glycol (0·3 c.c.), and the formic acid was titrated with 0·01N-sodium hydroxide (methyl-red). A blank experiment was performed simultaneously. The following figures represent the number of $C_6H_{10}O_5$ residues per mole of formic acid liberated: 73 hours, 37·9; 120 hours, 31·9; 168 hours, 30·1; 240 hours, 29·4; 380 hours, 29·1.

(iii) *Production of formaldehyde.* On application of the phenylhydrazine hydrochloride–potassium ferricyanide colour reaction to the solution of periodate-oxidised triticin, after destruction of periodate with sodium arsenite, a negative reaction was obtained.

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