Disaccharide Synthesis following Fructose-transfer from Sucrose by Yeast Invertase.

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The action of yeast invertase on a concentrated solution of sucrose containing D-glucose produces a disaccharide fraction containing 6-*O*-D-fructofuranosyl-D-glucopyranose. This substance was isolated contaminated by at least two difructoses, the structures of which were tentatively deduced from chromatographic and borate paper electrophoresis to be 6-*O*-D-fructofuranosyl-D-fructofuranose and 1-*O*-D-fructofuranosyl-D-fructose respectively.

Further evidence is thus provided in favour of the view that yeast invertase transfers fructofuranosyl (or fructofuranosido) radicals from the donor molecule, sucrose, to acceptors bearing primary alcoholic groups.

Bacon (*Biochem. J.*, 1954, **57**, 320; *Ann. Reports*, 1954, **50**, 281) has recently summarised and extended observations on fructose-transferring reactions by yeast invertase–sucrose systems made in his laboratory, and elsewhere, especially those of Bacon and Edelman (*Arch. Biochem.*, 1950, **28**, 467). The latter observed the formation of a chromatographically fast-running fraction, "Component I," a fructose-glucose disaccharide distinguishable from sucrose because it is reducing. Bacon (*loc. cit.*) has shown this substance to be a fructosylglucose since the glucose moiety can be oxidised by hypoiodite without affecting the hexulose component. Edelman (*Biochem. J.*, 1954, **57**, 22) has extended the original work by using [^{14}C] glucose and [^{14}C]fructose as acceptors.

Whelan and Jones (*Biochem. J.*, 1953, **54**, xxiv) have observed the formation of a similar disaccharide in the system, methyl β-D-fructofuranoside (fructose-radical donor); yeast invertase: D-glucose (fructose-radical acceptor). This disaccharide, like that of Bacon, had $[\alpha]_D$ +5° in H_2O; after reduction by sodium borohydride, oxidation by sodium metaperiodate of the product led Whelan and Jones to deduce its structure as 6-*O*-β-D-fructofuranosyl-D-glucose.

We now report that the fructosylglucose (Component I), formed by fructose transfer from sucrose as donor to glucose as acceptor, has the same structure as Whelan and Jones's compound. Use of Purdie's reagents (initially in methanol) yielded an octa-*O*-methyldisaccharide; chromatographic separation of its hydrolysis products on a silica column yielded 1 : 3 : 4 : 6-tetra-*O*-methyl-D-fructose and 2 : 3 : 4-tri-*O*-methyl-D-glucose, both substances being finally characterised as crystalline derivatives.

We regret that we were not in a position to commence our study of this fructosylglucose with a pure sample. Our starting material was contaminated by about 5% of "difructose." During the methylation preferential loss of fructosylglucose took place with consequent increase in the relative proportion of methylated "difructose." Although we were unable to obtain pure octamethylfructosylglucose, the chromatographic operations subsequent to methylation and hydrolysis of the starting material not only showed the presence of a single tri-*O*-methylglucose but also yielded information about the "difructose." Examination of the tri-*O*-methylfructose fraction ($[\alpha]_D$ −15° in H_2O) isolated from the hydrolysate indicated the presence of two sugars, almost certainly the 1 : 3 : 4- and the 3 : 4 : 6-trimethyl isomer.

The "difructose" is therefore a mixture of at least two components, 6-*O*-D-fructosyl-D-fructose and 1-*O*-D-fructosyl-D-fructose. It can be logically presumed that the glycosidic links are β. In the former case, the fructose acceptor-radical must be in the furanose form; this need not necessarily hold in the second case, however. It is of course possible that these fructosyl fructoses arose by hydrolysis of higher saccharides.

In conclusion, despite our failure here to effect a completely quantitative analysis, we consider that our results provide further evidence that by the action of yeast invertase fructofuranosyl (or fructofuranosido) radicals are transferred only to primary alcoholic groupings

of suitable acceptor molecules (cf. Bacon, *Biochem. J.*, 1952, **50**, xvii; Whelan and Jones, *loc. cit.*; Albon, Bell, Blanchard, Gross, and Rundell, *J.*, 1953, 24; Bacon and Bell, *J.*, 1953, 2528; Gross, Blanchard, and Bell, *J.*, 1954, 1727).

Experimental

Solutions were evaporated under reduced pressure below 40°.

Preparation of the Disaccharide Mixture.—Sucrose (100 g.), glucose (50 g.), dialysed B.D.H. invertase (10 ml.), and water (250 ml.) were incubated at 20° for 100 min. The mixture was then kept in a boiling-water bath for 10 min., then allowed to cool. Packed cells (60 g.) of *Candida krusei* (an invertase-free organism) were added to reduce the hexose content and the mixture incubated at 30° for 40 hr.; copious evolution of carbon dioxide resulted. After removal of solids on the centrifuge, the supernatant liquid was evaporated to a thick syrup; this was diluted with water to 200 ml., and an insoluble residue removed on the centrifuge. The supernatant liquid (60 ml.), which was neutral to bromothymol-blue, was run into a charcoal (NY3)–celite (1 : 1) column (36 × 17 cm.) (this column had previously been exhaustively washed with water until the effluent was neutral to bromothymol-blue). Gradient elution was started immediately with 25% (v/v) ethanol dropping into a continuously stirred reservoir-volume of 4 l. of water. A mixture of Component I and " difructose " emerged after the first 4·25 l. of eluate. The bulk of these sugars (detected by paper chromatography; Edelman, *loc. cit.*) emerged in the subsequent 430 ml. of eluate which was evaporated to a dry syrup. This was placed on a B.D.H. charcoal–celite (1 : 1) column (300 × 35 mm.) previously washed with water to give an effluent neutral to bromothymol-blue. Gradient elution (75% aqueous ethanol into 2 l. of water) gave some separation of the " difructose " from Component I; a considerable mixed fraction was, nevertheless, obtained. As much " difructose " as possible was eliminated in this way without discarding too much of Component I. Quantitative paper-chromatographic analysis (Bacon and Edelman, *Biochem. J.*, 1951, **48**, 114) showed the presence of 760 mg. of Component I and 90 mg. of " difructose," as assayed from their fructose contents.

Repetition of the charcoal chromatographic operation gave a syrup containing 500 mg. of Component I and 27 mg. of difructose. It had $[\alpha]_D^{20}$ +1·2° ±0·3°. As it was considered inadvisable to attempt further separations owing to the losses which occurred this material was examined by methylation as described below.

Methylation of Crude Component I.—To 500 mg. dissolved in dry methanol (10 ml.), 5 ml. of methyl iodide (dried over K_2CO_3) were added followed by silver oxide (20 g.) and calcium sulphate (5 g.). The whole was kept at room temperature with occasional shaking for 48 hr. Solids were removed by filtration and washed with hot, dry methanol (100 ml.). The combined filtrates, on evaporation, gave 450 mg. of a colourless syrup, strongly reducing towards Fehling's solution and insoluble in dry acetone. Two further treatments as above yielded 400 mg. of a non-reducing syrup, soluble in methyl iodide, which was subjected to two methylations with Purdie's reagents. The final product was a syrup which did not crystallise; it gave a single paper-chromatographic spot with a number of solvents and showed no evidence of containing monosaccharide derivatives (Found : OMe, 55·0. Calc. for $C_{20}H_{38}O_{11}$: OMe, 54·6%).

Preliminary Examination of the Methylated Material.—A sample, hydrolysed for 2 hr. with 0·02N-sulphuric acid at 100° gave, on a paper chromatogram, evidence (solvents, *n*-butanol–water; sprays, aniline hydrogen phthalate, and urea–hydrochloric acid) of 1 : 3 : 4 : 6-tetra-*O*-methylfructose and two tri-*O*-methylfructoses. No aldose could be detected; on the assumption that the glucose moiety of Component I has the pyranose structure and that its reducing group must have been methylated, a methyl glycopyranoside would not be detected by the aldose spray used.

Hydrolysis (2 hr. at 100°) with N-hydrochloric acid showed the presence of a trimethylhexose, with the same R_G as 2 : 3 : 4-tri-*O*-methylglucose, in addition to the methylated fructoses noted above.

Silica-column Chromatography of the Hydrolysed Methylated Material.—As a result of the paper-chromatographic experiments described above, the mixture (300 mg.) of methylated component I and methylated " difructose " was treated as follows: A solution in 0·02N-sulphuric acid was heated for 60 min. at 100°. The acid was then neutralised by barium carbonate and, after removal of solids by filtration through a bed of charcoal. the filtrate was concentrated to a syrup and transferred to a silica–water column made from 10 g. of dry silica

and 5 g. of water (Bell and Palmer, *J.*, 1949, 2522). After passage of 28 column-lengths of toluene containing 0·33% of ethanol through the column, and evaporation of the eluate, 260 mg. of material (Fraction I) were obtained.

The material remaining in the column was extracted by methanol; the solution, on evaporation, yielded ~20 mg. of syrup (Fraction II), $[\alpha]_D^{20}$ −15° (*c*, 0·7 in H_2O; *l*, 2), containing two trimethylhexuloses only, which occupied the paper-chromatographic positions of 1 : 3 : 4- and 3 : 4 : 6-tri-*O*-methylfructose. This mixture was examined by borate paper-electrophoresis (see below).

Fraction I, on paper chromatography, *appeared* to contain only 1 : 3 : 4 : 6-tetra-*O*-methylfructose. It was heated in N-hydrochloric acid (100 ml.) for 120 min. at 100°. The hydrolysate, after neutralisation with silver carbonate in the usual way, was concentrated to a syrup and this was transferred to a column made from silica (10 g.) and water (5 g.). Elution by 12 column-lengths of water-saturated chloroform, followed by evaporation of the eluate (cf. Bell, *J.*, 1944, 473), yielded 130 mg. of 1 : 3 : 4 : 6-tetra-*O*-methyl-D-fructose, $[\alpha]_D^{20}$ +30° in H_2O, n_D^{20} 1·4512 (cf. Bell, *J.*, 1953, 1231). Aldoses were absent. The substance gave "tetramethylfructofuronamide," m. p. and mixed m. p. 100—101° (Haworth, Hirst, and Nicholson, *J.*, 1927, 1513).

The eluted column was then extracted with methanol to yield, finally, 90 mg. of a colourless syrup of a single chromatographic component, an aldohexose, in the position of 2 : 3 : 4-tri-*O*-methylglucose. The syrup had $[\alpha]_D^{19}$ +79° in H_2O and n_D^{20} 1·4705 (cf. Greville and Northcote, *J.*, 1952, 1945); it gave an aniline derivative, m. p. and mixed m. p. 147—148° (cf. Peat, Schlüchterer, and Stacey, *J.*, 1939, 581).

Borate-paper Electrophoresis of Tri-O-methylfructose Fraction.—The mixed sugars of Fraction II were examined by Consden and Stanier's method (*Nature*, 1952, **170**, 1069) (cf. Bell and Dedonder, *J.*, 1954, 2866). Bell and Northcote (*Chem. and Ind.*, 1954, 1320) have recorded the electrophoretic behaviour on borate paper of a number of methylated fructoses and have demonstrated a distinction between those compounds which must, or can, exist in the furanose form and those which must have the pyranose structure, by means of Dedonder's urea–hydrochloric acid spray (*Bull. Soc. chim.*, 1952, **19**, 874). The former sugars give a blue-grey colour while the latter were stained ochre. When 1 : 3 : 4 : 6-tetra-*O*-methyl-D-fructose was used to mark the start-line and 3 : 4 : 6-tri-*O*-methyl-D-fructose as standard (distance migrated, 1·00), the following relative migration distances were found for the appropriate tri-*O*-methylfructoses: 1 : 3 : 4-, 0·0; 1 : 4 : 6-, 1·13; 1 : 4 : 5-, 0·96; Fraction II, 0·0 and 1·00. All the sugar spots were stained blue-grey, except that of 1 : 4 : 5-tri-*O*-methylfructose which gave the expected ochre.

Although 1 : 3 : 5-, 1 : 3 : 6-, and 3 : 4 : 5-tri-*O*-methyl-D-fructoses were not available, there can be little doubt that the two components of Fraction II were the 1 : 3 : 4- and the 3 : 4 : 6-derivative. 1 : 3 : 6-Tri-*O*-methyl-D-fructose would almost certainly not migrate (unless it passed into the keto-form); it would stain as a furanose derivative but would have a positive rotation so that a mixture with 3 : 4 : 6-tri-*O*-methyl-D-fructose would have also a positive rotation (~+30°). Fraction II had $[\alpha]_D$ −15° in H_2O. Both 1 : 3 : 5- and 3 : 4 : 5-tri-*O*-methyl-D-fructoses would be expected to be stained ochre and have large negative rotations.

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