877. *Synthesis of Sucrose Labelled with Carbon-14 in the Fructose Part.*

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A dextransucrase preparation from *Leuconostoc mesenteroides* (Birmingham) syntheses, from sucrose and D-[^{14}C]fructose, sucrose labelled in the fructose part, and other [^{14}C]fructose-containing saccharides.

It is generally accepted that the reaction between sucrose and dextransucrase to give dextran and D-fructose proceeds through several stages, involving association of the enzyme with donor and receptor molecules.[1,2] In the present work an investigation on the effect of added D-[^{14}C]fructose is described.

When dextransucrase preparations from *Leuconostoc mesenteroides* (Birmingham)[3] were incubated with sucrose and D-[^{14}C]fructose, radiochromatograms showed that carbon-14 was incorporated into four components, B, C, D, and E, which had, in solvent (*a*), $R_{fructose}$ values of 0·50, 0·38, 0·22, and 0·11, respectively. Components C, D, and E were produced only in relatively small quantities. This phenomenon was also observed with dextransucrase preparations from *Streptococcus bovis*, kindly supplied by Dr. R. W. Bailey. In the absence of any enzyme preparation, D-[^{14}C]fructose remained the only labelled compound in the mixture.

The following evidence shows that component B was in fact [^{14}C]sucrose: (*a*) in several solvents it migrated as a single component with an R_F value identical with that of sucrose; (*b*) hydrolysis by acid, and by invertase gave glucose and [^{14}C]fructose; (*c*) carrier dilution with sucrose gave crystalline [^{14}C]sucrose, the specific radioactivity of which was not affected by recrystallisation. The fact that acid and enzymic hydrolysis of [^{14}C]sucrose gave [^{14}C]fructose as the only component containing carbon-14 also showed that carbon-14 was present in the fructose portion only.

The specific radioactivity of the [^{14}C]sucrose present at the end of the incubation period (8 hr.) was 60·0 mc/mole by the infinitely-thin film method (Fig. 1), and 60·8 mc/mole by the infinitely-thick disc method. Based on the quantities (and radioactivity) of sucrose and D-[^{14}C]fructose present initially, a complete interchange of D-[^{14}C]fructose between sucrose and free D-fructose should give [^{14}C]sucrose with a specific radioactivity of *ca.* 62·0 mc/mole. The results show that this interchange is, within experimental error, virtually complete after 8 hours' incubation.

This investigation has shown that dextransucrase preparations are capable of transferring D-glucopyranosyl units reversibly from sucrose to the reducing position of D-fructofuranose. If the sucrose synthesising enzyme is in fact dextransucrase then the formation of a D-glucosyl-dextransucrase complex and D-fructose from sucrose is a reversible process. On the other hand, the synthesis of α-1,2- and α-1,6-linkages by our enzyme preparations[3] appears to be irreversible;[4] dextran synthesis is certainly very much slower than the D-fructose interchange.

The component C was, in several solvents, chromatographically identical with leucrose (5-*O*-α-D-glucopyranosyl-D-fructose). No hydrolysis occurred under mild acid conditions suitable for the complete hydrolysis of sucrose, or by treatment with invertase. More drastic conditions, however, gave glucose and a trace of [^{14}C]fructose, by chromatographic evidence. The conditions of this hydrolysis were such that most of the fructose would have been converted into hydroxymethylfurfuraldehyde, which would not have been detected in the chromatographic analysis. It is therefore suggested that component C was,

[1] Barker and Bourne, *Quart. Rev.*, 1953, **7**, 56.
[2] Bovey, *J. Polymer Sci.*, 1959, **35**, 191.
[3] Bailey, Barker, Bourne, and Stacey, *J.*, 1957, 3530.
[4] Bourne, Peters, and Weigel, unpublished results.

in fact, [^{14}C]leucrose; leucrose is known to be formed in this type of system.[5-7] The apparent ability of dextransucrase preparations to transfer D-glucopyranosyl units from sucrose also to C-5 of D-fructose is in agreement with our findings that six-membered ring compounds will act as receptors only if they possess two hydrogen atoms and one oxygen atom *cis* related on C-1, C-3, and C-5.[4]

The properties of components D and E suggest that they were produced by successive addition of glucosyl units to sucrose.

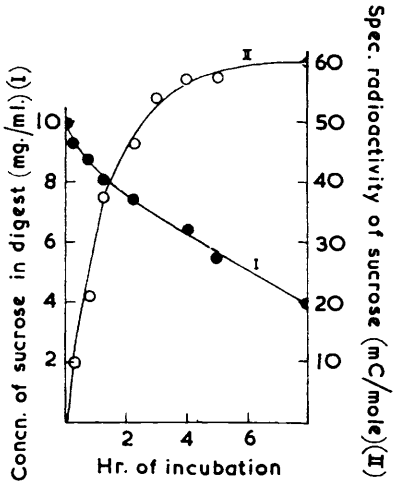
Experimental

Materials.—D-[^{14}C]Fructose, generally labelled, was obtained from the Radiochemical Centre, Amersham.

Determination of Radioactivity.—The apparatus and the methods used were those described previously.[8]

Chromatography.—(i) *Solvents.* The solvents used in chromatography were (*a*) butan-1-ol–ethanol–water (4 : 1 : 5) (organic phase); (*b*) ethyl acetate–acetic acid–water (9 : 1 : 1); (*c*) ethyl acetate–pyridine–water–acetone (10 : 5 : 10 : 2), to which ethyl acetate was added until two layers separated (organic phase).

(ii) *Radiochromatograms.* They were obtained by exposure of chromatograms to Ilford *X*-ray films (Industrial G) for an appropriate time.



Concentration and specific radioactivity of [^{14}C]sucrose during incubation of digest.

Oligosaccharide Synthesis in the Presence of D-[^{14}C]*Fructose.*—A digest containing sucrose (10 mg.), D-[^{14}C]fructose (*ca.* 100 mg.; spec. radioactivity 10·88 mc/g.-atom of carbon, *i.e.*, 65·28 mc/mole of D-fructose), and dextransucrase preparation (2·5 units) in 0·05M-acetate buffer (pH 5·0; 1 ml.) was incubated at 25° for 8 hr. The components of portions (0·05—0·1 ml.), withdrawn at intervals, were fractionated by paper chromatography in solvent (*a*). This revealed, in addition to D-[^{14}C]fructose, A, four components containing carbon-14, B, C, D, and E. The components B and C had R_{fru} values identical with those of sucrose (0·50) and leucrose (0·38), respectively. The R_{fru} values of components D and E were 0·22 and 0·11, respectively.

Specific Radioactivity of Component B at Time Intervals.—Each fraction containing component B, obtained by chromatographic fractionation, was diluted with water to 25 ml. Portions (1 ml.) were analysed for carbohydrate content by the anthrone method.[6] The specific radioactivity of component B in each fraction was determined by the infinitely-thin film method [8] and expressed per mole of disaccharide. The results are shown in the Figure.

Characterisation of Component B.—(i) In each of the three solvents used component B moved as a single radioactive and chemical component with an R_F value identical with that of sucrose.

[5] Stodola, Koepsell, and Sharpe, *J. Amer. Chem. Soc.*, 1952, **74**, 3202.
[6] Bailey and Bourne, *Nature*, 1959, **184**, 904.
[7] Bourne, Hutson, and Weigel, *Biochem. J.*, 1961, **79**, 549.
[8] Bourne, Hartigan, and Weigel, *J.*, 1959, 2332.

(ii) *Hydrolysis.* A sample of component B was hydrolysed with 0·25N-sulphuric acid a 100° for 15 min. Another sample of component B was incubated with invertase (B.D.H. concentrate) at 20° for 1 hr. Chromatographic analysis of the hydrolysates revealed in each case components which had R_F values identical with those of glucose and [^{14}C]fructose.

(iii) *Carrier dilution.* Sucrose (255·7 mg.) was dissolved in a portion of the fraction containing component B (0·22 mg.) obtained after incubation of the digest for 8 hr. After being freeze-dried, the sucrose was crystallised from dry methanol and propan-2-ol. Recrystallisation did not cause a decrease in specific radioactivity (52·26 μc/mole, by infinitely-thick disc method [8]). From this the specific radioactivity of component B at the end of the incubation period was calculated (60·8 mc/mole of disaccharide).

Investigation of Component C.—(i) In each of the three solvents used component C moved as a single radioactive and chemical component with an R_F value identical with that of leucrose.

(ii) Samples of the combined fractions of component C were (*a*) heated with 0·25N-sulphuric acid at 100° for 15 min. and (*b*) incubated with invertase (B.D.H. concentrate) at 20° for 1 hr. Radiochromatograms revealed only a single radioactive component identical with component C. Another sample was heated with 1·5N-sulphuric acid at 100° for 4 hr. Chromatography of the hydrolysate revealed the presence of glucose and a trace of a component containing carbon-14 and having R_F identical to that of fructose.

Investigation of Components D and E.—Samples of components D and E were separately heated with 1·5N-sulphuric acid at 100° for 4 hr. Chromatography revealed in each case components corresponding to glucose and [^{14}C]fructose (trace). Chromatographic analysis of partial hydrolysates (N-sulphuric acid; 90°; 1 hr.) revealed in each case the presence of components which had R_F values identical with those of isomaltose, glucose, and [^{14}C]fructose.

Incubation of Sucrose with D-[^{14}*C*]*Fructose.*—Sucrose (10 mg.) and D-[^{14}C]fructose (100 mg.) dissolved in 0·05M-acetate buffer (pH 5·0; 1 ml.) were kept at 25° for 25 hr. Radiochromatograms of the solution revealed only a single radioactive component with R_F identical to that of fructose.

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