Oligosaccharides Formed from Sucrose by Fructose-transferring Enzymes of Higher Plants

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The synthesis of oligosaccharides by the transfer of hexose residues from one molecule of sucrose to another has been observed with invertase preparations from yeast and filamentous fungi (see summary by Bacon, 1954*a*; also Gross, Blanchard & Bell, 1954; Bell & Edelman, 1954). The hexose residue transferred is fructose, resulting in the formation of oligosaccharides enriched in fructose. Fructose may also be transferred to other acceptor molecules with primary-alcohol groups, including a variety of sugars and alcohols (Bealing, 1953; Bacon, 1954*b*). Free glucose serves as an acceptor with both invertase preparations, though the disaccharides formed are different with yeast and mould enzymes (Edelman, 1954). Purification and other treatments of an invertase preparation lead to parallel changes in the hydrolytic and fructose-transferring activity, indicating that the two types of action are due to a single enzyme. An analogous transferring action has been observed in extracts of *Helianthus tuberosus*, but the enzyme involved is probably different from invertase, since inulin and oligosaccharides of the inulide series are preferential substrates for this enzyme (Edelman & Bacon, 1951; Dedonder, 1952*b*).

The products formed by yeast invertase acting on sucrose have been found to differ quantitatively and qualitatively from those produced by invertase from filamentous fungi, but some of the products are common to both types of enzyme. Table 1 gives some information about two of the latter; in the account that follows their 'abbreviated' names will be used. 1^F-β-Fructosylsucrose was crystallized by Barker, Bourne & Carrington (1954) and

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Table 1. *Trisaccharides from the action of invertase preparations*

Structure	Source of invertase	$[\alpha]_D$	Authors	Trivial names	Abbreviated names
O-α-D-Glucopyranosyl-(1→2)-*O*-β-D-fructofuranosyl-(1→2) β-D-fructofuranoside	Taka diastase	+27°*	Bacon & Bell (1953)	Component α_1 (Bealing & Bacon, 1953); 1-Kestose (Gross *et al.* 1954)	1^F-β-Fructosyl-sucrose
	Penicillium altramentosum	+29·0	D. Hibbert (personal communication, 1954)	—	—
	Aspergillus niger	+29·2	Barker *et al.* (1954)	Trisaccharide I	—
	Yeast	+27*	Bacon (1954*b*)	Component II_1	—
	Helianthus tuberosus†	+23*	Dedonder (1952*a*)	Glucofructosane B	—
	H. tuberosus†	+28·6	J. S. D. Bacon (unpublished, 1954)	Spot 2 (Bacon & Edelman, 1951)	—
	Beta vulgaris	+28·6	Present work	—	—
O-β-D-Fructofuranosyl-(2→6)-α-D-glucopyranosyl-(1→2) β-D-fructofuranoside	Yeast	+21*	Bacon (1954*b*)	Component II_2	6^G-β-Fructosyl-sucrose
	Yeast	+22*	Gross *et al.* (1954)	*neo*Kestose	—
	Beta vulgaris	+21*	Present work	—	—

* Measured on an amorphous preparation.

† In *H. tuberosus* the substance is a normal constituent of the carbohydrates of the tuber; the invertase of this plant has not been investigated.

D. Hibbert (personal communication), but 6^G-β-fructosylsucrose is still available only in the amorphous state.

In the present paper, data are presented concerning the fructose-transferring properties associated with invertase activity in extracts from higher plants. It will be shown that two trisaccharides are the main products of fructose transfer by an enzyme preparation from the leaves of sugar beet (*Beta vulgaris* L.) and that these trisaccharides have properties identifying them with the two trisaccharides mentioned above.

METHODS

Enzyme preparations. An active enzyme preparation from sugar-beet leaves was obtained by squeezing the juice from fresh tissues through bolting cloth in a hydraulic press at pressures up to 2000 lb./in.^{2}. The petiole and main veins were dissected out and the juice was expressed separately from these tissues and from the remaining leaf blade. The juice was stored in Pyrex flasks at $-10°$ and did not change in invertase or in fructose-transferring activity during 10 months' storage. Samples were used either without further purification or after removal by centrifuging of material which precipitated during freezing and thawing. For some experiments the enzyme was slightly enriched and purified by partial freezing, decanting and dialysing in the cold against distilled water.

Other enzyme preparations were obtained by grinding tissue with a mortar and pestle and expressing the juice with the pestle, or by crushing frozen tissues in a Hughes press (Hughes, 1951). Grinding with sucrose did not detectably increase the activity of extracts. Prior treatment of leaves with ether vapour resulted in a greatly increased yield of juice with considerably reduced invertase activity.

Incubation of samples. Incubations were carried out at room temp. in stoppered flasks. The final concentration of substrate was usually 20% (w/v). For incubations of more than a few hours, $CHCl_3$ was added to prevent microbial growth. The pH values of the juices used ranged from 5·0 to 6·5. When adjustment of the pH was made, acetate buffer was employed. Before chromatograms of the reaction products were developed, the enzyme was inactivated with $HgCl_2$ placed on the starting line of the paper (Bealing & Bacon, 1953).

Measurement of radioactivity. This was carried out essentially as described by Edelman (1954). All papers were sprayed with benzidine–trichloroacetic acid (Bacon & Edelman, 1951) shortly before counting, and values obtained from the two sides of the paper were averaged.

Autoradiograms. These were prepared by placing the paper chromatograms in close contact with envelope-packed Ilford Industrial G X-ray Film (Ilford Ltd., Ilford, London) for periods of 10–40 days. The films were then developed for 6 min. in a D 19 B X-ray film developer (Kodak Ltd.) and fixed with an acid hardener. Papers which had been sprayed gave autoradiograms indistinguishable from those from unsprayed papers.

Paper chromatography. The apparatus and procedures were those described by Bacon & Edelman (1951). *n*-Butanol–acetic acid–water (Partridge, 1948), and Whatman no. 1 paper were used throughout. For routine detection of carbohydrates, the dried papers were sprayed with benzidine–trichloroacetic acid. The reducing properties of sugars were compared with those of suitable controls, chromatographed under the same conditions, by the use of the alkaline $AgNO_3$ reagents of Trevelyan, Procter & Harrison (1950) (see Bealing & Bacon, 1953; Edelman & Bacon, 1951).

Column chromatography. The products formed from sucrose after incubation with sugar-beet-leaf juice were separated by gradient elution from charcoal–Celite columns with increasing concentrations of ethanol (Alm, Williams & Tiselius, 1952) and examined according to the procedures described by Bacon (1954*b*).

Analytical procedures. Ketose was determined colorimetrically as described by Bacon & Bell (1948). Total reducing sugar before and after hydrolysis was determined by the method of Miller & Van Slyke (1936) with fructose as a standard (Bacon, 1954*b*). The reducing-sugar composition and optical rotation of the oligosaccharides were measured as described by Bacon & Edelman (1951). Melting-point determinations were made in capillary tubes inserted into an air space surrounded by an electrically heated jacket.

RESULTS

Separation and purification of two trisaccharides

When an extract of sugar-beet-leaf blades was incubated at pH 6·0 with 20% sucrose, chromatograms of the incubation mixture showed spots appearing in the glucose and fructose positions within a few hours and increasing in intensity for several days. After 6 hr. incubation, a third spot appeared behind the sucrose position, with an R_S (quotient R_F sugar/R_F sucrose) of approximately 0·6, and increased in intensity during the following week. On the basis of its R_S, this spot appeared to represent a trisaccharide. Usually a very faint spot also appeared after a day or two between this new component and sucrose. This spot, with an R_S value of about 0·75, never became very conspicuous and tended to disappear before the trisaccharide.

Extracts from sugar-beet petioles showed similar products after longer incubation with sucrose, but extracts from the root were almost completely lacking in activity. Hydrolysis of sucrose and formation of trisaccharide appeared to be as fast at pH 5·0 as at 6·0, but were both slower at pH 4·0.

To obtain material for further study of these products, 95 ml. of leaf-blade juice, 40 g. of A.R. sucrose, and 45 ml. of 0·2 M sodium acetate–acetic acid buffer, pH 5·0, were mixed (total vol. 160 ml.) and incubated in the presence of $CHCl_3$ for 8 days, during which time approximately half the sucrose was broken down. On centrifuging the mixture, a clear supernatant was obtained, which was fractionated on charcoal–Celite columns. A preliminary gradient elution of a 20 ml. sample resolved the mixture into four fractions. Paper chromatograms showed these to consist, in order of their appearance, of (1) glucose and fructose,

(2) sucrose, (3) and (4) two distinct components representing the single spot in the trisaccharide position. Of these two components, the one eluted first was present in large amount and had a slightly lower R_S value; a typical chromatogram, carrying three spots of each, gave mean R_S values of 0·606 and 0·620 for the earlier and later components respectively. In addition to these five sugars, trace amounts of two other sugars appeared between sucrose and the major trisaccharide.

The incubation mixture (125 ml.) was chromatographed on a B.D.H. 'activated' charcoal–Celite column (300 g.:300 g.; about 90 cm. high, 5·5 cm. in diam.), the eluates being collected on solid $BaCO_3$. The two trisaccharides were sharply separated. Each sample was filtered, evaporated to dryness *in vacuo* at below 40°, dissolved in 5·0 ml. of water, rechromatographed on Sutcliffe Speakman Active Carbon no. 130–Celite (50 g.: 50 g.), and the purified material again evaporated to dryness. The residues were further dried over $CaCl_2$ at 0·1 mm. Hg for 2 days, and weighed. At this stage, yields of 611 and 133 mg. respectively were obtained from about 31 g. of sucrose present in the original 125 ml. of incubation mixture. Each sample was again dissolved in an accurately measured quantity (5·0 ml.) of glass-distilled water, and the resulting solutions were used for further study as such, or after further purification.

Attempts to crystallize these sugars were successful with the major but not with the minor component. About 0·5 g. of the former was dissolved in 2 ml. of dry methanol and left in a sealed desiccator containing ethanol. The next day a non-crystalline precipitate had separated from the mixture of methanol and ethanol. Upon seeding with crystals of 1^F-β-fructosylsucrose (kindly provided by Mr D. Hibbert) the material crystallized. It was later recrystallized by dissolving in methanol with a trace of water and crystallizing as before, yielding 310 mg. of fine white crystals.

The major trisaccharide. On the basis of its reaction with the alkaline $AgNO_3$ and phloroglucinol sprays, this compound was non-reducing and contained ketose. The recrystallized material did not react to the Raybin (1933) test, which is essentially, though not exclusively, a test for derivatives of sucrose in which the fructose moiety is unsubstituted (see Levi & Purves, 1949). Upon slow hydrolysis with acetic acid (1 vol. of acetic acid added to 5 vol. of 10·8 %, w/v, solution of trisaccharide) two products appeared early and increased in concentration together. These two corresponded in position on paper chromatograms with sucrose and fructose. A third product, appearing later in the hydrolysis, corresponded chromatographically with glucose. No other products were detected at any stage. These results are consistent with a structure for the trisaccharide in which an extra fructose residue is linked to the fructose moiety of sucrose. The glucose which arose during the later stages of the hydrolysis could have come either from the sucrose liberated earlier or from direct hydrolysis of the trisaccharide, in which case a fructosylfructose would have been formed; this substance, which has almost the same R_F value as sucrose, was not looked for.

Analysis of the original syrup, and of the recrystallized material, for ketose content indicated a ratio of 2 ketose:1 non-ketose residue, when calculated on the basis either of dry weight or of total reducing sugars after hydrolysis with 0·5 % oxalic acid at 100° for 25 min. (Table 2).

The $[\alpha]_D^{20}$ of the recrystallized sugar, based on dry weight, was 28·6° in water; based on ketose content it was 29·1°. Its melting point was sharp at 200° before and after recrystallization. Decomposition occurred rapidly at this temperature after the crystals had melted, but there was no evidence of decomposition before melting.

Dr D. Gross has examined this substance by electrophoresis in borate buffer (see Gross, 1954; 4000 v, 46 mA, 2 hr.); it has the same mobility as 1^F-β-fructosylsucrose.

The minor trisaccharide. Like the major trisaccharide, this component was a non-reducing ketose-containing sugar. It gave a positive Raybin test, however. Slow hydrolysis with acetic acid led to the immediate appearance and rapid accumulation of sugars chromatographically identical with fructose and sucrose and of a third component travelling between the sucrose and the parent trisaccharide positions in *n*-butanol–acetic acid–water. This substance was a reducing sugar, which could arise from a non-reducing trisaccharide containing a sucrose residue only by splitting of the 1:2-glycosidic linkage between glucose and fructose. Since its accumulation preceded the appearance of glucose, hydrolysis of the 1:2-linkage must not have released free glucose. It therefore seemed probable that this reducing disaccharide was a fructosylglucose containing a fructose residue transferred during the original incubation with the leaf juice. That the substance contained both sugars was confirmed as follows: three spots of the partially hydrolysed trisaccharide were chromatographed, after which the paper containing the outer spots was cut out and sprayed to locate the position of the reducing disaccharide on the unsprayed portion. This was then cut out and eluted on to the starting line of a second paper with a concentrated solution of yeast invertase, according to the method of Zimmermann (1953). The second paper was developed, and sprayed with alkaline $AgNO_3$. Hydrolysis of the disaccharide had occurred during elution, and comparable amounts

Table 2. *Analysis of trisaccharide fractions*

Preparation	Sample taken (mg.)	Total reducing substance (mg.)	Ketose (mg.)	Ketose:non-ketose, on basis of		Purity, on basis of		$[\alpha]_D^{20}$ on basis of	
				Dry wt.	Total reducing substance	Total reducing substance (%)	Ketose* (%)	Dry wt.	Ketose†
Major trisaccharide									
(i) Syrup, original preparation	183·3	182·6	115	2:1·42	2:1·18	93·0	87·8	25·7°	29·0° (12·22)
(ii) (i) filtered and re-dried	324·5	314·4	201·5	2:1·45	2:1·12	90·5	86·9	25·8	29·4 (10·82)
(iii) Recrystallized	102·1	116·0	71·6	2:1·06	2:1·24	106	98·3	28·6	29·1 (2·04)
Minor trisaccharide									
(i) Syrup, original preparation	177·4	177·8	107·8	2:1·53	2:1·30	93·5	85·1	20·9	24·6 (4·43)
(ii) (i) diluted 1·5 ×	—	—	—	—	—	—	—	22·0	25·8 (1·96)
(iii) (ii) filtered and re-dried	80·5	84·3	56·0	2:1·08	2:1·01	97·7	97·4	20·6	21·2 (1·77)

* Assuming composition 1 aldose:2 ketose.
† Figure in parentheses is concn., as % (w/v), of solution used for measuring rotation.

of two reducing sugars were produced, running in the fructose and glucose positions. This fragment of the trisaccharide is probably identical with the fructosylglucose (component I) formed during the action of yeast invertase on sucrose (Bell & Edelman, 1954).

Ketose determinations yielded values of approximately 2:1 for the ketose:non-ketose ratio in this trisaccharide also (Table 2). The $[\alpha]_D^{20}$ of the purest preparation obtained was 20·6° in water on a dry-weight basis, 21·2° on the basis of ketose content.

The infrared absorption spectrum of a preparation made by precipitating the sugar from methanolic solution with ether was examined by Drs S. A. Barker and E. J. Bourne, and compared with that of 6^G-β-fructosylsucrose. They report: 'The trisaccharide has a similar, but not identical, infrared spectrum to that of [6^G-β-fructosylsucrose]. The main difference is that [6^G-β-fructosylsucrose] shows an extra peak at 888 cm.$^{-1}$.'

Dr D. Gross has examined this trisaccharide electrophoretically under the same conditions as the major trisaccharide and finds that it has the same mobility as 6^G-β-fructosylsucrose.

Action of sugar-beet-leaf enzymes on various oligosaccharides

The action of the leaf extract at pH 6·0 was tested against 20% (w/v) solutions of maltose, melezitose, trehalose, and the major trisaccharide described above, and against 10% (w/v) solutions of cellobiose and raffinose and a 20% (w/v) suspension of soluble starch, and qualitative comparisons were made with the activity against sucrose. On paper chromatograms, no detectable products were formed from starch or melezitose during 6 days' incubation. From maltose and from cellobiose traces of glucose and of a trisaccharide were formed. Trehalose, raffinose, sucrose and the trisaccharide were acted on much more rapidly than the other oligosaccharides. The only product appearing from trehalose was glucose. From the trisaccharide, substances travelling in the fructose and sucrose positions were first evident, later glucose and a new substance with R_S 0·30, probably a tetrasaccharide. This trisaccharide can thus serve both as substrate and acceptor for the enzyme. Raffinose yielded four products, all of which were clearly evident after 1 day. Three of these were chromatographically identical with fructose, glucose and galactose respectively. The fourth had an R_S of 0·62 and contained no ketose residue. It was therefore probably melibiose, which would be left upon removal of fructose from the raffinose molecule. Some transferring action to raffinose was indicated by the appearance at later stages of a spot in the tetrasaccharide position. Only traces of sucrose appeared at any stage in the incubation.

Transfructosylation to free glucose

Since raffinose was split chiefly by removal of fructose, and very little sucrose accumulated, the possibility presented itself of determining directly whether glucose might serve as a fructose acceptor with consequent synthesis of sucrose. Incubations of a dialysed leaf-juice preparation containing 10% (w/v) of raffinose, with or without 10% (w/v) of glucose, showed no evidence of component I and only traces of sucrose, which was formed equally whether or not glucose was present. No significant transfer from raffinose to glucose was indicated.

The question was further investigated by experiments to determine whether radioactivity became incorporated into sucrose or component I when sucrose was incubated with the enzyme in the presence of [^{14}C]glucose (cf. Edelman, 1954). A difficulty was encountered in these experiments: the chromatograms of uniformly labelled [^{14}C]glucose (obtained from the Radiochemical Centre, Amersham) showed some radioactivity in the di- and tri-saccharide positions. This amounted to about 2% of the total counts from the paper; autoradiograms showed it to consist partly of an unspecific trail, and partly of discrete spots, one of which might correspond with isomaltose/gentiobiose (Bacon & Bacon, 1954). When the experiments of Edelman (1954) with Taka diastase were repeated the incorporation of radioactivity into sucrose was found to be of such a magnitude that this contamination could be disregarded, but preliminary experiments with the sugar-beet enzyme indicated that incorporation, if any, was much less, and was obscured by the 'blank'.

After discussions with Dr Edelman, who had encountered similar difficulties, the following procedure was adopted: ten spots, each of 2·5 μl. of [^{14}C]glucose in 2% glucose solution (a solution made by dissolving 1·3 mg. of [^{14}C]glucose, having 100 μc radioactivity, in 0·5 ml. of 2% (w/v) glucose), were disposed at intervals of 0·5 cm. along the starting line of a strip of paper 17·5 cm. wide.

A guide spot of inactive glucose was placed 3·5 cm. away from either end and the chromatogram developed with *n*-butanol–acetic acid–water overnight (15–20 hr.). The paper was dried in a current of air at 50°, and strips carrying the guide spots were cut out, leaving the radioactive material on a strip 10·5 cm. wide. After the guide strips had been sprayed with benzidine–trichloroacetic acid, the chromatogram was reassembled and two lines, about 1·5 cm. apart, were drawn across the sheet within the fringes of the guide spots, but including the greater part of the glucose. The strip so formed was cut out, a point cut at one end, and elution with water begun in the open air of the laboratory after the manner of a descending paper chromatogram. Evaporation from the strip was such that no free water accumulated at the tip. After 0·5–1·0 hr. under these conditions the apparatus was covered by an inverted jar; a drop of water began to accumulate at the point of the paper. In some cases it was necessary to place some damp filter paper in the jar to bring this drop to the size at which it would fall off. Measurement of radioactivity showed that a drop (25–30 mg.) produced in this way contained 90% or more of the glucose applied to the chromatogram; it was essential to allow evaporation during the elution, a drop collected by elution in a humid atmosphere containing only about 10% of the glucose.

In a typical experiment 25 mg. of sucrose was dissolved in 0·025 ml. of 0·2M sodium acetate–acetic acid buffer, pH 5, and a drop (29 mg.) of the purified [^{14}C]glucose eluate added, followed by 0·15 ml. of the sugar-beet enzyme preparation. Samples of 5 μl. were chromatographed on paper and the distribution of radioactivity was studied by direct counting of successive 0·5 cm. levels of the chromatogram, and by autoradiograms. Table 3 shows the changes in the radioactivity of the sucrose position during incubation at 20°. (From the appearance of chromatograms sprayed with benzidine–trichloroacetic acid it was estimated that between one-half and three-quarters of the sucrose had been broken down in 145 hr.) Autoradiograms (3 or 5 μl. spots exposed for 3–5 weeks) showed the presence of four radioactive spots in the di- and tri-saccharide regions. One of these coincided exactly with the sucrose spot as revealed by the benzidine–trichloroacetic acid spray. Another, with R_F value somewhat smaller than that of sucrose, coincided with a very faint spot revealed by the

Table 3. *Incorporation of radioactivity into sucrose during incubation of sugar-beet-leaf enzyme preparation with sucrose and* [^{14}C]*glucose*

Reaction mixture (details in text) incubated at room temp. in Agla micrometer syringe. Radioactivity expressed as counts/min. from paper carrying the equivalent of 5 μl. of incubation mixture.

Time (hr.) ...	0*	1·7	4·5	28	47	99	145
Sucrose	0	3	7	17	22	35	32
Glucose	6281	—	6328	—	—	—	—

* Actually 10 min. No significant radioactivity was counted anywhere on this chromatogram apart from the glucose spot; 5 μl. of the reaction mixture placed on paper, but not run, gave 6434 counts/min.

spray. This radioactive spot appeared with equal intensity in autoradiograms of incubation mixtures in which the sucrose was replaced by fructose; it did not appear when the enzyme preparation was incubated with radioactive glucose alone. It therefore seems possible that this corresponds to component I. The remaining two radioactive spots did not correspond with any spots revealed chemically; they lay above and below the trisaccharide spot, in which there appeared to be negligible radioactivity, as would be expected if it were produced from the very feebly labelled sucrose. They may have been formed by fructose transfer from sucrose to the hypothetical radioactive component I, which would be expected to be of high specific activity.

Radioactive sucrose was formed only when sucrose was the substrate; in incubations with radioactive glucose alone the glucose remained throughout the only spot revealed by autoradiograms.

Transfructosylation with other plant juices

Oligosaccharide formation from sucrose was catalysed by juices extracted from the leaves, shoots and roots of other plants, and was always related in intensity to the invertase activity of the preparation. (The preparation of highest activity produced visible trisaccharide in 3 hr.) The extracts examined and showing formation of upper spots included those from leaves of broad bean (*Vicia faba* L.), thistle (*Cirsium arvense* Scop.), cow parsnip (*Heracleum sphondylium* L.), elder (*Sambucus nigra* L.), coltsfoot (*Tussilago fafara* L.), white clover (*Trifolium repens* L.), cabbage (*Brassica oleracea* L.), and mangel-wurzel (*Beta vulgaris* L.), and from young shoots of thistle and root tips of broad bean; an active extract was also made from the only monocotyledon examined, wheat (*Triticum vulgare* Vill.). Extracts without invertase or oligosaccharide-forming activity were obtained from the roots of sugar beet and mangel-wurzel, the cotyledons of broad bean, from apple fruit and the leaves of ivy (*Hedera helix* L.). The last two extracts were more acid than the others (pH 2–3 instead of 5·0–6·5) and the ivy-leaf extract was very viscous.

In all preparations the only conspicuous spot formed had an R_S value comparable with that of the trisaccharides from sugar-beet-leaf preparations. Traces of higher oligosaccharides were formed in some wheat-leaf preparations, and from cow parsnip and broad bean there was a transient and slight production of another sugar, running between the trisaccharide and sucrose positions.

DISCUSSION

The evidence presented here shows that an invertase preparation from sugar-beet leaf has transfructosylase activity, and that in the presence of sucrose the main product of transfer is the trisaccharide 1^F-β-fructosylsucrose, already known as a product of the action of yeast and mould invertases, and a normal constituent of the Jerusalem-artichoke tuber. The other trisaccharide is probably 6^G-β-fructosylsucrose, although a final identification must await the crystallization of this substance or a derivative.

Qualitative experiments with other higher plants suggest that the sugar-beet enzyme is typical. The differences between the three types of fructosaccharases are summarized schematically in Fig. 1. Like yeast invertase, the invertase of higher plants shows considerably more hydrolytic than transferring action.

In view of the suggestion (cf. Kurssanov, 1941) that invertase is part of the system responsible for the synthesis of sucrose in leaves, we have investigated the possibility that sucrose might be formed by fructose transfer to free glucose. Edelman & Bealing (1953) showed that mould invertase catalyses a rapid incorporation of radioactive glucose into sucrose. Yeast invertase, on the other hand (Edelman, 1954), catalyses its incorporation into a different fructosylglucose, probably 6-β-fructosylglucose (Bell & Edelman, 1954).

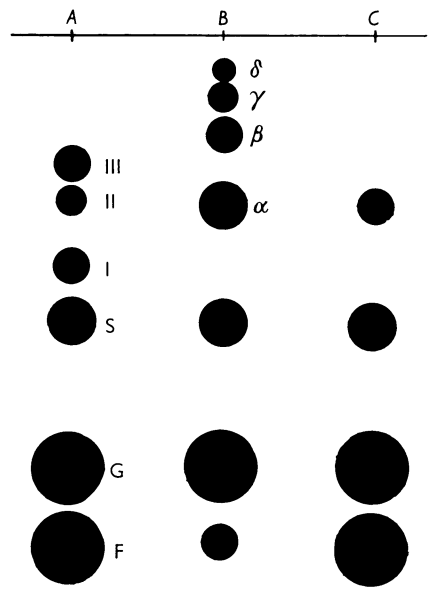

Fig. 1. Diagrammatic representation of intermediate stages in the breakdown of sucrose by invertase preparations from various sources (*A*, yeasts; *B*, moulds; *C*, higher plants), as revealed by paper chromatography in *n*-butanol–acetic acid–water. The sizes of the spots indicate approximately the amounts of the various compounds present. The lettering of the spots is according to Bacon & Edelman (1950) for yeast invertase, and Bealing & Bacon (1953) for mould invertase.

With the sugar-beet-leaf preparation the incorporation of radioactivity into the sucrose position of chromatograms was so slight that it was not practicable to confirm it by isolation of sucrose. However, the autoradiograms and the results of control experiments suggest that incorporation into sucrose is taking place, and that it is not due to a simple reversal of hydrolysis. By comparison with the mould enzyme, the activity is so small that it is difficult to believe that it can have any physiological significance. It is possible that yeast invertase may also have the ability to form sucrose to this extent, because the experiment described by Edelman (1954, Table 8) does not exclude incorporation of the magnitude found in our experiments.

Unlike the invertase of filamentous fungi, the preparations from higher plants did not form significant amounts of tetra- or higher oligosaccharides. Some other explanation must therefore be sought for the higher fructose-containing oligosaccharides found in such monocotyledons as wheat (White & Secor, 1953), barley (Porter & Edelman, 1952) and grasses (Laidlaw & Reid, 1952), or in the Compositae (Bacon & Edelman, 1951; Dedonder, 1952*a*). On the other hand, if due precautions are not taken to inactivate the invertase, trisaccharides may arise as artifacts in plant extracts.

SUMMARY

1. The action of aqueous extracts of higher plants on sucrose, and on other di- and tri-saccharides, was studied by paper chromatography. In all cases hydrolysis of sucrose was accompanied by the formation of trisaccharides.

2. The trisaccharides produced from sucrose by an enzyme preparation from the leaves of sugar beet were isolated and tentatively identified as *O*-α-D-glucopyranosyl-(1 → 2)-*O*-β-D-fructofuranosyl-(1 → 2) β-D-fructofuranoside and *O*-β-D-fructofuranosyl-(2 → 6)-α-D-glucopyranosyl-(1 → 2) β-D-fructofuranoside.

3. During the action of the sugar-beet-leaf enzyme preparation on sucrose in the presence of uniformly labelled [^{14}C]glucose some incorporation of radioactivity into the di- and tri-saccharide fractions was detected.

4. The significance of these results is discussed in relation to the hypothesis that leaf invertase participates in sucrose synthesis.

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