BIOCHEMISTRY

Formation of Aryl β-D-Fructofuranoside by Enzymatic Transglycosylation

In the enzymatic transglycosylation reactions catalysed by ordinary glycosidases, the transfer of glycosyl group from aryl glycosides to simple alcohols and sugars was known to proceed easily[1], whereas the reverse reaction, namely, with alkyl glycosides and oligo- and poly-saccharides as donor and phenols as acceptor, has not been realized. These facts suggest that the bond-energy of aryl glycosides may be higher than that of alkyl glycosides or oligosaccharides, so the failure of aryl glycoside formation from alkyl glycosides or oligosaccharides might be due to the insufficiency of the bond energy of the latter. For the biosynthesis of aryl glycoside the intervention of uridine diphosphate sugars has been shown to be needed[2]. In view of the comparatively high bond energy of sucrose it appeared not unlikely to carry out enzymatic transfer of fructosyl residue of sucrose to phenols leading to the formation of aryl fructoside. The present report describes the enzymatic formation of *p*-hydroxyphenyl β-D-fructofuranoside from sucrose and hydroquinone and its characterization.

The purification procedure of yeast invertase was based mainly on acetone precipitation and chromatography on 'Duolite *C*-10'[3]. The purified invertase solution was free of most other carbohydrases including α-glucosidase. Fructose and fructoside were estimated colorimetrically by the method of Roe *et al.*[4] after separation by paper chromatography.

A solution containing sucrose (7 per cent) and hydroquinone (8 per cent) was incubated with purified yeast invertase preparation at *p*H 6·0 for 90 min and the products were separated by paper chromatography with a solvent mixture of *n*-butanol/ethanol/water (100 : 15 : 30). On the chromatogram a new spot with R_F 0·57 was recognized besides the spots corresponding respectively to hydroquinone, sucrose, glucose, fructose and oligosaccharides. By the colour reaction of the new spot the product was shown to be composed of fructose and hydroquinone. In control experiments, where either sucrose was replaced by equimolar solution of glucose and fructose or boiled enzyme was used, no such spot was detected. This new substance made its appearance at an early stage of sucrose hydrolysis, gradually increasing in amount, but it disappeared completely before all the sucrose was hydrolysed. These findings would indicate that the substance may be a fructoside of hydroquinone formed by the enzymatic transfer of β-fructofuranosyl group from sucrose to hydroquinone. Several lines of experiments such as fractionation and partial inactivation of the enzyme preparation as well as inhibition study suggest that the invertase itself would be responsible for the formation of the fructoside.

With the invertase preparations from taka-diastase and wheat ear the formation of similar fructoside was also observed.

The isolation of the transfer product was carried out by the use of column chromatography with charcoal–'Celite' and paper chromatography. From 10 l. of the reaction mixture 541 mg of crude crystal of the product was obtained. Repeated recrystallization from water gave 235 mg of pure colourless platelet crystal.

The substance, which had been dried at 26°–28° and 0·1 mm Hg showed m.p. 119° and $[\alpha]_D$ −119·8° (water, c. 2) and gave following values of elementary analysis: found, C 51·10, H 5·89, calculated for $C_{12}H_{16}O_7 \cdot 1/2H_2O$, C, 51·23; H, 6·08. It was considerably stable in alkali, but very unstable in acid. Treatment with 0·1 N sulphuric acid at 30° for 5 h or with 0·5 per cent oxalic acid at 100° for 20 min completely hydrolysed the substance into fructose and hydroquinone in equimolar proportion. The substance was easily hydrolysed by every invertase preparation from different sources as shown in Table 1.

Table 1. Hydrolysis of β-D-Fructofuranosides by Several Invertase Preparations

Reaction mixture contained 6·25 μmoles substrate, 50 μmoles acetate buffer, *p*H 4·7, and enzyme solution in a total of 0·5 ml. Incubated at 30°. Results are expressed as relative ease of hydrolysis with the activity for sucrose taken as 100.

Source of enzyme \ Substrate	Sucrose	Hydroquinone fructofuranoside	Methyl fructofuranoside
Brewers' yeast	100	350	9
Taka-diastase	100	370	12
Wheat ear	100	190	15

An infra-red spectrum of the substance showed absorption peaks at 928 cm^{-1}, 875 cm^{-1}, 857 cm^{-1}, 820 cm^{-1}, and 986 cm^{-1}, characteristic of the furanose ring structure[5], in addition to several peaks corresponding to *p*-hydroxyphenyl derivatives, but no absorption was observed indicative of the existence of pyranose ring structure[6]. All these findings agree well with the postulated structure of the compound, *p*-hydroxyphenyl β-D-fructofuranoside.

The achievement in the isolation of this compound is significant in that this is the first aryl glycoside synthesized by the transglycosylative activity of the hydrolytic glycosidase and that this is the only aryl β-D-fructofuranoside so far obtained.

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Molecular Weight of Sheep Pituitary Interstitial Cell-stimulating Hormone

Two new procedures for the isolation of interstitial cell-stimulating hormone (ICSH) from sheep pituitary glands have been described in recent years[1,2]. Sedimentation coefficients ($s_{20°,w}$) of the hormone were reported to be 2·47 in phosphate buffer[1] of *p*H 6·8 and 2·32 *S* in glycine buffer[2] of *p*H 6·0. From these values, together with values for the diffusion coefficient and partial specific volume, these authors[1,2] computed the molecular weight of ICSH to be 28,000–30,000. We have recently re-examined the sedimentation behaviour of ovine ICSH and found that the molecular weight of the hormone changes with the *p*H of the buffer. Results also indicate that the ICSH molecule forms reversible aggregates. We wish to report herein the molecular weight of the ICSH monomer.

Sedimentation experiments were performed in a Spinco model *E* analytical ultracentrifuge, equipped with Rayleigh optics. Analysis of sedimentation equilibrium data is based on the equation of Svedberg and Pedersen[3] as previously described[4]. The hormone was dissolved in potassium chloride–hydrochloric acid buffer of *p*H 1·3 and ionic strength 0·1. When the logarithm of the concen-