COMMUNICATIONS TO THE EDITOR

SYNTHESIS OF SUCROSE AND OTHER β-D-FRUCTOFURANOSYL ALDOSIDES BY LEVANSUCRASE

Sir:

Analogs of sucrose are formed when a bacterial transglucosidase is allowed to act on glucosyl donor (sucrose) in the presence of appropriate ketoses serving as acceptors.[1] The present communication reports a complementary property in the levansucrase system,[2] namely, the catalysis of a process in which the aglycone of β-D-fructofuranosyl aldosides is transferred reversibly to the anomeric carbon position of an aldose.

When a cell-free solution of levansucrase of *Aerobacter levanicum*[2] was allowed to act on raffinose, there were formed levan, fructose, and melibiose but neither glucose nor galactose. In the presence of added D-glucose, raffinose with levansucrase formed little levan but there occurred a rapid formation of a non-reducing disaccharide which behaved like sucrose on paper chromatograms. In absence of glucose this disaccharide was not formed. The disaccharide was isolated as a chromatographically pure compound by band paper chromatography. The facts that the material was completely hydrolyzed to the component sugars, D-glucose and D-fructose, by yeast invertase, and was converted to levan, D-fructose and D-glucose by levansucrase further characterized the substance as sucrose.

Neither dextransucrase[3] by itself nor a mixture of dextransucrase and levansucrase formed dextran from raffinose alone. When this system was supplemented with glucose, rapid formation of dextran occurred. Since melibiose but not galactose was released from raffinose by the levansucrase, it is suggested that levansucrase formed sucrose from raffinose and glucose, and that this disaccharide was then converted by dextransucrase to dextran.

When levansucrase was allowed to act on sucrose in the presence of melibiose, a non-reducing oligo saccharide with the paper chromatographic mobility of raffinose was found to be formed. These findings indicate that levansucrase catalyzes the establishment of an equilibrium of the following form:

$$\text{Raffinose} + \text{Glucose} \rightleftarrows \text{Sucrose} + \text{Melibiose} \quad (1)$$

It has been found that many aldoses are able to react with raffinose in this way in the presence of levansucrase. For example, the interaction of raffinose and D-xylose has afforded a non-reducing disaccharide which has been isolated as a chromatographically pure compound by means of gradient elution from a carbon column. The isolated compound ($[\alpha]^{20}D$ +62°) was readily hydrolyzed totally to xylose and fructose by yeast invertase, and was found to be converted by levansucrase to levan, fructose and xylose. Periodate oxidation afforded 1 mole formic acid per mole disaccharide. These and other properties[4] suggest that the compound is a sucrose analog, α-D-xylopyranosyl-β-D-fructofuranoside, for which the name "xylsucrose" may be appropriate. The following aldoses have now been shown to be converted to corresponding aldosyl-β-D-fructofuranosides on reaction with raffinose or sucrose in the presence of levansucrase: D-xylose, L-arabinose, D-glucose, D-galactose, and melibiose.

A unit reaction in the elongation of a levan chain by the action of levansucrase can be pictured in the light of these results as the sum of a primary reversible and of a subsequent irreversible step (equations 2 and 3)

$$\text{fr} \sim \text{R} + \text{enz} \rightleftarrows \text{fr} \sim \text{enz} + \text{R} \quad (2)$$

$$\text{fr} \sim \text{enz} + \text{fr}_n \longrightarrow \text{fr}_{n+1} + \text{enz} \quad (3)$$

where equation 2 shows the reversible transfer of the fructofuranosyl group (fr) from a donor molecule (fr ~ R) to the enzyme (enz) with release of aldose (R), and equation 3 shows the irreversible reaction in which a levan chain (fr_n) of n anhydrofructose residues is increased to $n + 1$ residues.

(1) W. Z. Hassid and M. Doudoroff, *Adv. Carboh. Chem.*, **5**, 29 (1950).

(2) S. Hestrin and S. Avineri-Shapiro, *Biochem. J.*, **38**, 2 (1944).

(3) E. J. Hehre, *Adv. Enzym.*, **11**, 297 (1951).

(4) G. Avigad, D. S. Feingold and S. Hestrin, *Biochim. et Biophys. Acta*, in press (1956).

LABORATORY OF MICROBIOLOGICAL CHEMISTRY OF THE DEPARTMENT OF BIOCHEMISTRY
HEBREW UNIVERSITY–HADASSAH MEDICAL SCHOOL
JERUSALEM, ISRAEL
SHLOMO HESTRIN
DAVID S. FEINGOLD
GAD AVIGAD

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STRUCTURAL STUDIES ON STREPTONIVICIN,[1] A NEW ANTIBIOTIC

Sir:

Streptonivicin[2] (I) the new antibiotic elaborated by *Streptomyces niveus* has the approximate formula $C_{30-32}H_{38-42}N_2O_{11}$ (calcd. for $C_{31}H_{42}N_2O_{11}$: C, 60.18; H, 6.85; N, 4.53. Found: C, 59.69; H, 6.66; N, 4.48). It is isolated from fermentation broths by acid precipitation or by solvent extraction from neutral or acid solution. Two crystal forms of I were obtained, melting at 152–156° (dec.)[3] and 174–178° (dec.), respectively. These polymorphs show equal optical activity [$[\alpha]^{24}D$ −63° (c, 1% in ethanol)] and identical ultraviolet absorption, with maxima in 0.01 N ethanolic (70%) sulfuric acid at 334 mμ (a = 40.7), in 0.01 N ethanolic (70%) phosphate buffer (pH 7.5) at 248 mμ (a = 36.2) and 308 mμ (a = 32.8) and in 0.01 N ethanolic (70%) potassium hydroxide at 311 mμ (a = 53.2). Rast determinations indicated a molecular weight of about 610 and X-ray crystallographic studies indicated 618. Potentiometric titrations, as well

(1) The Upjohn Company Registered Trademark (U. S. Patent Office) for stretonivicin is Albamycin.

(2) (a) Feng-Kai Lin and Lewis L. Coriell, paper delivered to the Third Annual Symposium on Antibiotics, Nov. 4, 1955, Washington, D. C.; (b) William J. Martin, *et al.*, Proc. Staff Meetings Mayo Clinic, in press; (c) Charles G. Smith, *et al.*, *Antibiotics and Chemotherapy*, in press; (d) Herman Hoeksema, *et al.*, *ibid.*, in press; (e) J. R. Wilkins, *et al.*, *ibid.*, in press.

(3) All melting points determined on a Kofler micro-hot-stage.