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).

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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.

as ultraviolet spectra, show I to be a dibasic acid (pK'_as 4.3 and 9.1 in water) with an equivalent weight of 636. The formation of neutral and acid salts with many bases confirms this. At least one methoxyl and two C-methyl groups are present. Catalytic hydrogenation of I with Adams (PtO_2) or Raney nickel catalysts yields dihydrostreptonivicin (II), m.p. 163–165° (calcd. for $C_{31}H_{44}N_2O_{11}$: C, 59.98; H, 7.15; N, 4.41. Found: C, 59.84; H, 6.44; N, 4.49). The potentiometric titration and optical activity of II are similar to those of I. The ultraviolet spectra are also similar, but the 334 mμ absorption of peak of I is found at 328 mμ for II. The infrared spectra of these compounds show similarities in the regions of carbonyl and conjugated system absorptions. The biological activity of II is very similar to that of I.

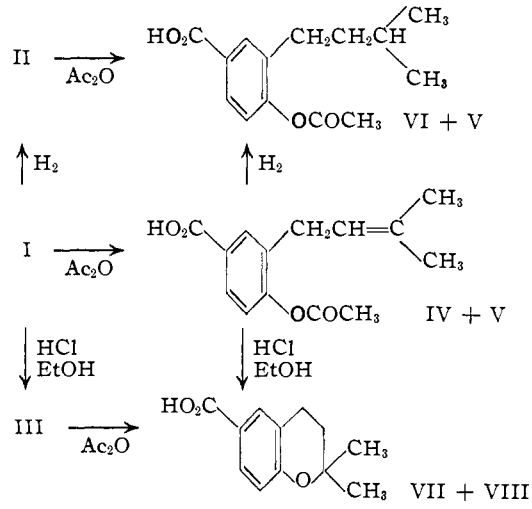
Hydrolysis of I by 4 *N* hydrochloric acid in 60% ethanol yields III, an optically inactive acid (pK'_a 6.3 in 66% dimethylformamide, m.p. 288–290° (dec.)) in 95% yields (calcd. for $C_{22}H_{21}NO_6$: C, 66.82; H, 5.35; N, 3.54. Found: C, 67.18; H, 5.40; N, 3.70). Compound III has characteristic ultraviolet absorption maxima in 0.01 *N* ethanolic acid at 330 mμ (a = 61.0) and in 0.01 *N* ethanolic potassium hydroxide at 251 mμ (a = 84.7) and 328 mμ (a = 68.2). The Kuhn–Roth determinations show the presence of at least one C-methyl group; no methoxyls are present.

Each of these three compounds is cleaved in refluxing acetic anhydride. From I is obtained an optically inactive acid (IV) (m.p. 116–120°, pK'_a 5.67 in 50% ethanol) and an optically active neutral compound (V) m.p. 167–173°, $[\alpha]^{24}D$ −94.4° (c, 2% in dimethylformamide). Calcd. for $C_{14}H_{16}O_4$ (IV): C, 67.72; H, 6.50. Found: C, 67.75; H, 6.56. The approximate formula $C_{23-25}H_{28-34}N_2O_{10-11}$ is assigned to V (calcd. for $C_{23}H_{28}N_2O_{10}$: C, 56.09; H, 5.73; N, 5.69. Found: C, 56.65; H, 5.44; N, 5.74. The cleavage of II yields V and an optically inactive acid (VI), m.p. 138–144° (calcd. for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.45; H, 6.93). From III are obtained 2,2-dimethyl-6-carboxychroman[4] (VII) and an optically inactive neutral compound (VIII) melting at 203–206° (calcd. for $C_{12}H_{14}O_3$ (VII): C, 69.88; H, 6.84. Found: C, 69.80; H, 6.71. Calcd. for $C_{14}H_{11}NO_5$ (VIII): C, 61.54; H, 4.06; N, 5.13. Found: C, 61.59; H, 3.77; N, 5.06). The identity of VII was established by comparison of melting points of VII and of its *p*-bromophenacyl ester with the published values, as well as by infrared and ultraviolet comparison of VII with an authentic sample graciously supplied by Prof. W. M. Lauer.

Catalytic hydrogenation of IV yields VI. Treatment of IV with alcoholic sodium hydroxide deacetylates it to a dibasic acid (IX) melting at 103–106°, with pK'_as of 6.2 and 11.0 in 66% ethanol (calcd. for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 69.89; H, 6.94). The characteristic ultraviolet absorption spectrum of IX is consistent with that of a substituted *p*-hydroxybenzoic acid, the substituent in this case would be a pentenyl side-chain in which the double bond is not conjugated with the ring.

(4) Walter M. Lauer and Owen Moe, THIS JOURNAL, **65**, 289 (1943).

The nature and position of the pentenyl group was deduced as follows: A band at 842 cm.$^{-1}$ in the infrared spectra of IV and IX was consistent with the presence of an isopropylidene group. Successive oxidations of IV with osmium tetroxide and sodium periodate yielded acetone as a major product. Finally, IV was converted to VII upon heating in 4 *N* hydrochloric acid in 70% ethanol. The structure 4-acetoxy-3-(3-methyl-2-butenyl)-benzoic acid was assigned to IV, 4-acetoxy-3-(3-methylbutyl)-benzoic acid to VI, and 4-hydroxy-3-(3-methyl-2-butenyl)-benzoic acid to IX. The linkage of this moiety (IX) to the rest of the streptonivicin molecule is through its carboxylic acid function.



Additional structural features of I will be published at a later date.

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A SYNTHESIS FOR 3,5-DIARYL-1-(5′-TETRAZOLYL)-(1H)TETRAZOLIUM BETAINES

Sir:

We wish to report the synthesis of a new class of tetrazolium betaines which are derived from (1H)-tetrazole.[1] All of the tetrazolium salts which have previously been reported have been prepared, directly or indirectly, by the oxidation of 1,3,5-trisubstituted formazans.[2] This oxidative ring closure affords only derivatives of (2H)tetrazole.[3]

It was demonstrated by Busch and Pfeiffer that treatment of a 1,3-diaryltetrazene with an aromatic aldehyde produces a 1,3,5-triarylformazan instead of the expected aldimine derivative of tetrazene.[4] This phenomenon was explained in terms of a facile tetrazene–formazan rearrangement. A

(1) The designation "(1H)" indicates that the salt is formed by the quaternation of a 1-substituted tetrazole.

(2) For an excellent discussion of this subject see A. W. Nineham, *Chem. Revs.*, **55**, 385 (1955).

(3) The preparation of a (1H)tetrazolium betaine has recently been reported by S. Hunig and O. Boes, *Ann.*, **579**, 28 (1953). However, the structure is not known with certainty.

(4) M. Busch and H. Pfeiffer, *Ber.*, **59**, 1162 (1926).