33. *Structural Studies of Dextrans. Part II.*[1] *The Enzymic Synthesis of 3-α-Isomaltosylglucose from Nigerose.*

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Potato T-enzyme acts on nigerose to produce glucose, isomaltose, and a trisaccharide shown to have the structure *O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucose. This trisaccharide is identical with one obtained on partial acetolysis of dextran NRRL 1355-S.

The preceding paper describes the isolation from a partly acetolysed dextran (NRRL 1355-S) of a glucose trisaccharide believed to have the structure *O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucose (3-α-isomaltosylglucose). It occurred to us that a potato enzyme (T-enzyme) recently described from this laboratory [2] might be able to synthesize this trisaccharide. T-Enzyme is a transglucosylase capable of redistributing α-1,6-linkages in the isomaltodextrins. Thus, isomaltose is converted reversibly into a mixture of isomaltotriose and glucose. Further transfer reactions from the products ensue. The action of the enzyme is not, however, confined to α-1,6-glucosidic bonds because it also splits the α-1,4-bond of maltose and transfers a glucose residue to the non-reducing glucose unit of a second maltose molecule, synthesizing an α-1,6-bond between the two sugars and creating the trisaccharide panose (4-α-isomaltosylglucose). The purpose of the present work was to test whether a similar reaction would occur with nigerose (α-1,3-glucobiose) as substrate, to give, by analogy with panose, 3-α-isomaltosylglucose. A trisaccharide thought to have this structure had already been synthesized from nigerose by a transglucosylase from *Aspergillus oryzae*,[3] but the structure was not rigidly defined.

A small-scale digestion of nigerose obtained from the dextran showed by paper chromatography that a trisaccharide was synthesized, and in a larger-scale experiment with 450 mg. of nigerose there were obtained glucose, isomaltose, and, after repeated fractionation on paper, a trisaccharide (25 mg., 8%) having the same mobility on a paper chromatogram as 3-α-isomaltosylglucose from dextran. The following evidence shows that the trisaccharide had the expected structure and confirms the assignment of the same structure to the dextran trisaccharide. (*a*) After reduction with sodium borohydride the synthetic sugar lost one-third of its capacity to liberate glucose on total acid hydrolysis.[4] The sugar was therefore a trisaccharide. (*b*) Partial acid hydrolysis of the sugar gave substances migrating on a paper chromatogram with the same mobility as isomaltose, nigerose, and glucose. (*c*) The trisaccharide migrated more rapidly during paper electrophoresis than did panose. The respective M_G values were 0·47 and 0·30. (*d*) The borohydride-reduced trisaccharide was oxidised with dilute sodium metaperiodate under conditions known to cause degradation only of the polyol end group.[5] Approximately 2·0 molecular proportions of formaldehyde were formed, the same amount as from reduced nigerose. The amounts of periodate consumed were consistent with selective end-group oxidation (see Experimental section).

According to the evidence in (*d*) the reducing-end linkage of the trisaccharides must be a 1,3- or a 1,4-bond, since only these structures can yield 2 mol. of formaldehyde. All other types of reducing-end linkage would yield only 1 mol. of formaldehyde. The M_G value of the trisaccharide (*c*) eliminates the possibility that the reducing-end linkage was a 1,4-bond. When the evidence of partial hydrolysis (*b*) is added, the only possible

[1] Part I, Goldstein and Whelan, preceding paper.
[2] Abdullah and Whelan, *Biochem. J.*, 1960, **75**, 12P.
[3] Pazur, Budovich, and Tipton, *J. Amer. Chem. Soc.*, 2957, **79**, 625.
[4] Peat, Whelan, and Roberts, *J.*, 1956, 2258.
[5] Clancy and Whelan, *Chem. and Ind.*, 1959, 673.

structure for the trisaccharide is 3-α-isomaltosylglucose. The assignment of the α-configuration to each of the two bonds is justified by the magnitude of the specific optical rotation of the trisaccharide, namely, $[\alpha]_D^{20}$ +150° in water, and the results of partial hydrolysis. Panose (4-α-isomaltosylglucose) has $[\alpha]_D$ +154° and isomaltotriose (6-α-isomaltosylglucose) $[\alpha]_D$ +142°.

These results confirm the structure expected of the trisaccharide as a result of its enzymic synthesis from nigerose. That this trisaccharide was identical with that obtained by fragmentation of dextran NRRL 1355-S [1] was shown by the identity of the crystalline undeca-acetates of the two sugars.

The course of synthesis of the trisaccharide may therefore be expressed as:

$$\text{Nigerose} + \text{Nigerose} \longrightarrow \text{3-}\alpha\text{-Isomaltosylglucose} + \text{Glucose} \quad . \quad . \quad (1)$$

The isomaltose also found when nigerose was incubated with T-enzyme could arise if glucose acts as the sugar receptor instead of nigerose, thus:

$$\text{Nigerose} + \text{Glucose} \longrightarrow \text{Isomaltose} + \text{Glucose} \quad . \quad . \quad . \quad . \quad (2)$$

It could also be formed as a result of transfer between the products of reaction (1). As stated above, the action of T-enzyme in converting maltose into panose is not, apparently, reversible. The α-1,6-bond, once formed, is not reconverted into an α-1,4-bond. If the analogy holds in the present case the " reversal " of reaction (1) would result in the formation of nigerose and isomaltose, the α-1,6-bond of the trisaccharide persisting as such on transfer to glucose.

Experimental

General Methods.—These are detailed in Part I of this series.[1] T-Enzyme was prepared from potatoes as by Abdullah.[6]

Synthesis of 3-α-Isomaltosylglucose.—To a solution of nigerose (0·450 g.) in 0·5M-sodium citrate buffer (pH 6·3; 15 ml.) was added a solution of T-enzyme (15 ml.; from 400 ml. of potato juice) which had been dialysed free from reducing carbohydrates. The digest was kept at room temperature for 48 hr. in the presence of toluene (1 ml.). The enzyme was inactivated by heating the digest for 5 min. at 100°. The coagulated protein was filtered, then washed thoroughly with water, and the buffer salts were removed with Biodeminrolit resin.[7] A paper chromatogram showed the presence in the digest of sugars migrating with the mobility of glucose (R_g 1·0), nigerose (R_g 0·69), isomaltose (R_g 0·47), a sugar having R_g 0·33 (referred to as trisaccharide), and a substance having R_g 0·16, which was not examined further. The mixture of sugars was separated by chromatography on thick filter paper into glucose (33·5 mg.), $[\alpha]_D$ +54° (*c* 0·33 in H_2O), isomaltose (15 mg.), $[\alpha]_D^{20}$ +125° (*c* 0·154 in H_2O), nigerose (300 mg.), and trisaccharide (45 mg.). The trisaccharide was contaminated with isomaltose and was further fractionated on paper to give a chromatographically pure syrup (25 mg.), $[\alpha]_D^{20}$ +150° (*c* 0·83 in H_2O). The results of partial acid hydrolysis of the trisaccharide in 0·33N-sulphuric acid for 30 min. at 100° are stated in the Discussion section. The trisaccharide (10 mg.) was reduced with sodium borohydride (20 mg.) for 24 hr. When it was hydrolysed with 1·5N-sulphuric acid for 6 hr. the glucose liberated amounted to 65% of that from an equivalent weight of unreduced trisaccharide. The sugar alcohol (4·39 mg., 0·7 ml.) was mixed with 20mM-sodium metaperiodate (4 ml.) and immediately diluted to 200 ml. with water. The solution was kept at room temperature and the production of formaldehyde was measured at intervals as by Clancy and Whelan.[5] Nigeritol (4·39 mg.), similarly formed from nigerose, was oxidized in the same way. The molecular proportions of formaldehyde produced were: trisaccharide alcohol, 4·25 hr., 1·75; 24 hr., 2·08; nigeritol, 3·6 hr., 1·93; 5 hr., 1·96. Periodate consumption was measured by adding 20 ml. portions of the digest to a mixture of 2·5% potassium iodide (10 ml.) and 3N-sulphuric acid (2 ml.) and titrating the iodine with 10mN-sodium thiosulphate. The molar proportions of periodate consumed at the times stated were trisaccharide alcohol, 3·00, 5·10; nigeritol, 3·14, 3·44.

[6] Abdullah, Ph.D. Thesis, London, 1960.
[7] Woolf, *Nature*, 1953, **171**, 841.

The trisaccharide (10 mg.) was acetylated with sodium acetate–acetic anhydride to give the crystalline undeca-acetate, m. p. 114—116°, which separated when the mixture was poured into water. On recrystallization from ethanol, this had m. p. 117—119° and mixed m. p. 117—120° with the acetate of the dextran trisaccharide.[1] The acetate had $[\alpha]_D$ +121° in chloroform (*c* 0·095). Potassium bromide discs of this acetate and that derived from dextran were examined for infrared absorption in a Perkin-Elmer Infracord spectrophotometer over the range 3—15 microns. The positions of peak absorption were identical.

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