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Isolation of Nigerose and Kojibiose from Dextrans

The occurrence of 1,3-α linkage in some dextrans has already been suggested by many workers[1–5]. Barker *et al.*[3,4] have fractionated the partial acid hydrolysate of the dextran produced by *Betacoccus arabinosaseous* (Birmingham strain) on a charcoal column and recognized the presence of a trace of nigerose by paper chromatography. Jeanes *et al.*[5] have characterized dextrans from 96 strains of bacteria by periodate oxidation analysis and classified them into several groups on the basis of the proportions of 1,3 like linkages. According to their results, 29 of them appeared to contain more than 10 per cent 1,3-linkages and a few of them showed an extremely high content of 1,3-linkages (more than 30 per cent).

In 1956, Scott *et al.*[6] pointed out the presence of 1,2-linkage in some dextrans from the optical rotational shifts due to formation of a cuprammonium complex. According to their report, a few of them (for example, that from *Leuconostoc mesenteroides* NRRL *B*–1299 or *B*–1399) contain more than 30 per cent 1,2-linkages.

In spite of these observations, however, no oligo- or di-saccharides containing the heterogeneous glucosidic linkages have so far been isolated from the hydrolysates of dextrans. This may be due to the difference of the stability of 1,6- and other linkages against acid hydrolysis; the 1,6-linkage is much more stable than the other linkages. Therefore, by the usual method of hydrolysis in aqueous acid solution, heterogeneous linkages are more rapidly hydrolysed than the 1,6-linkage.

We have now found that acetolysis is more effective for the isolation of the heterogeneous linkages from dextrans. In contrast with hydrolysis, the 1,6-linkage is less stable to acetolysis, whereas the other linkages such as 1,2- and 1,3- are more resistant. For example, when the acetates of 1,6-linked isomaltose and 1,3-linked nigerose were subjected to acetolysis using the method to be described, the former was entirely cleaved into glucose in 6 hr. at 25° C., whereas the latter remained unchanged. By this method, we have now been able to isolate nigerose and kojibiose from the acetolysates of some dextrans. The dextrans used were prepared by the usual method from *Leuconostoc mesenteroides* NRRL *B*–421 and *B*–1299. The acetolysis reagent was a mixture of acetic anhydride, glacial acetic acid and concentrated sulphuric acid (24:16:3 by vol.). 20 gm. of dried and powdered dextran was suspended in 172 ml. of the above acetolysis reagent and kept at 30° C. for 7 days. The reaction mixture was then heated on a water-bath at 80° C. for 30 min. and poured into crushed ice. After neutralization with sodium carbonate, the whole solution was extracted with chloroform. On removal of the solvent, syrupy mixed acetates were obtained, which were deacetylated with sodium methoxide as usual. Yields of the acetolysates were 10–15 gm. from 20 gm. of the original dextrans. To ascertain if a reversible reaction had occurred, anhydrous glucose was treated exactly as described above; but no reversion product was detected by paper chromatography. The acetolysate of the dextran from *Leuconostoc mesenteroides* NRRL *B*–421 (29·7 gm.) was fractionated on a charcoal/'Celite' column by the gradient elution method. From the 5 per cent ethanol effluent, 7·8 gm. of nigerose and only 0·4 gm. of isomaltose were obtained. Nigerose was characterized as its crystalline octaacetate, melting point 149° C., undepressed on admixture with an authentic specimen.

In the same manner, 7·5 gm. of kojibiose and 1·0 gm. of nigerose were obtained from 22·0 gm. of the acetolysate of the dextran of *Leuconostoc mesenteroides* NRRL *B*–1299. Kojibiose was also characterized as its α- and β-octaacetates: α-kojibiose octaacetate, melting point 166° C.; β-kojibiose octaacetate, melting point 118° C. Neither acetates showed any depression on admixture with authentic specimens.

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