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Note

Purification of inulobiose obtained by acid hydrolysis of inulin

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Inulobiose (1-*O*-β-D-fructofuranosyl-D-fructose) was required in a pure state for mass spectrometry. Preparation of the disaccharide by partial acid hydrolysis of inulin (according to methods previously described[1,2]) yielded a product, isolated by paper chromatography, which gave 7.5% of D-glucose on acid hydrolysis. Inulobiose has an R_F value very close to that of sucrose, and it was reasoned that the D-glucose must have arisen from contaminant sucrose.

Inulin has a d.p. of ~34 and the terminal residue is an α-D-glucopyranose residue linked to a β-D-fructofuranose residue as in sucrose, the remainder of the molecule being composed of (2→1)-linked β-D-fructofuranose residues[3]. Since the rate constants for acid hydrolysis of inulobiose and sucrose, under the conditions described, are 0.018 and 0.042 min^{-1}, respectively[1], the ratio of sucrose to inulobiose in a partial hydrolysate of inulin should reduce to ~1:7, *i.e.*, a D-glucose content of 6.3%.

Paper electrophoresis of the impure inulobiose in a borate buffer removed the contaminant sucrose (migration rates towards the anode: sucrose −1.7, inulobiose +13.0 cm/h at 2 kV and 230–240 mamp). The ratio of the weights of the sucrose to inulobiose thus obtained was 1:6. The difference between this value and the theoretical ratio, together with the higher D-glucose content of the unresolved mixture (see above), suggests that the d.p. of the original inulin sample may have been <34. Samples of crude inulin, also tested, gave still higher ratios of sucrose to inulobiose.

Purified inulobiose had m.p. 189–192.5°, $[\alpha]_D^{20}$ −66 ±0.5° (water), yielded no glucose on hydrolysis, and had a fructose content of >99%. The yield from 2 g of inulin was 25 mg.

The differences between the specific rotation of the purified product first recorded[1] and the values subsequently found[2,4] could partly be ascribed to the presence of sucrose in the first preparations. Moreover, the lower rotation of the samples prepared here suggests that some earlier preparations[2,4] may possibly have been contaminated with fructose.

EXPERIMENTAL

Inulin (2 g; Sigma Chemical Co.) was hydrolysed for 30 min at 70° in 10 ml of 0.01M HCl; the resulting mixture was cooled and neutralised with sodium carbonate[1]. Alternatively, some hydrolysates were neutralised by passage through a column of Amberlite IR-4B(HO^-) resin; on final purification, the inulobiose derived from these samples had a slightly higher ($\sim$1°) negative rotation. The neutralised solutions were concentrated to small volume under reduced pressure at 50°, and then subjected to chromatography[5] on Whatman 3MM paper using 7:1:2 and 6:1:3 1-propanol–ethyl acetate–water and 100:22:50 1-butanol–acetic acid–water in the descending mode. Fructosides were detected with urea–metaphosphoric acid[6].

Electrophoresis was carried out in borate buffer[7]. The borate complexes derived from the electrophoresis were decomposed by evaporation of solutions at 40° in 9:1 methanol–water containing sufficient acetic acid to keep the pH at 3.5–4.0. Residual borate and other ions were removed by passing the product through Amberlite IR-120(H^+) and IR-4B(HO^-) resins. This last treatment was only carried out on samples that had previously been through the resin.

Total hydrolysis of the disaccharides was carried out in M H_2SO_4 for 2 h at 100°; the hydrolysates were deionised by using resins as described above.

Fructose in solution was determined by the method of Cole (see ref. 8), and free D-glucose by the D-glucose oxidase method[9].

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