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Note

A practical preparation of *p*-nitrophenyl β-D-mannopyranoside*

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p-Nitrophenyl β-D-mannopyranoside (**2**) is a useful substrate for β-D-mannopyranosidase[1], an enzyme essential for structural studies of many glycoproteins. Although **2** was once commercially available, it is not at present marketed, because of difficulties in production. Presumably, **2** should be obtainable from the reaction of penta-*O*-acetyl-α,β-D-mannopyranose (**1**) with *p*-nitrophenol under the conditions described by Helferich[2]. Nevertheless, isolation of **2** from the reaction mixture, largely consisting of its α anomer (**3**), seemed difficult.

Recently, we have reported an easy chromatographic separation of anomeric pairs of several aryl glycopyranosides[3], and this technique was successfully employed to prepare some aryl 1,2-*cis*-1-thioglycopyranosides[4]. We now describe another application of this technique, namely, to isolate **2** (7–8%) from the Helferich reaction-products rich in **3** (60–65%).

The reaction conditions are simple, and the chromatographic separation uses a column of a commonly available ion-exchange resin (Dowex 50) eluted only with water; thus, this procedure can be readily utilized in any laboratory. An added advantage of this method is that a large amount of **3** is obtained simultaneously as a "by-product". As most investigators concerned with β-D-mannopyranosidase must also deal with α-D-mannopyranosidase activities, such a "by-product" should be a welcome additional benefit.

EXPERIMENTAL

Condensation of p-*nitrophenol with penta*-O-*acetyl*-α,β-D-*mannopyranose* (**1**). — Compound **1**† (5 g; 12.8 mmoles), recrystallized *p*-nitrophenol (5.35 g, 38.5 mmoles),

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†Prepared by acetylation of D-mannose with acetic anhydride–pyridine; it consisted of 20% of β and 80% of α anomer.

and freshly fused zinc chloride (0.26 g) were weighed into a 50-ml Erlenmeyer flask with a magnetic stirring bar. The flask was kept for 30 min in an oil bath (140°) with vigorous and efficient stirring*. During the reaction, the mixture was continuously flushed with a stream of nitrogen. The flask was then removed from the oil bath, and after cooling, the reaction products were dissolved in benzene (250 ml) and the solution was successively washed with 1M sodium hydroxide (five times) and water (twice), dried (sodium sulfate), and evaporated. The residue was dissolved in a small volume of toluene, and the solution evaporated to ensure complete removal of water.

Deacetylation and chromatographic separation of the reaction products. — The dried reaction-products were deacetylated in dry methanol (200 ml) with 7.6 meq of sodium methoxide for 3.5 h at 4°. The deacetylation was monitored by t.l.c. on silica gel (Merck, F_{254}) with 1:1 (v/v) benzene–ether and 9:4:2 (v/v/v) ethyl acetate–isopropyl alcohol–water. After decationization with Rexyn 101 (H^+), the methanolic

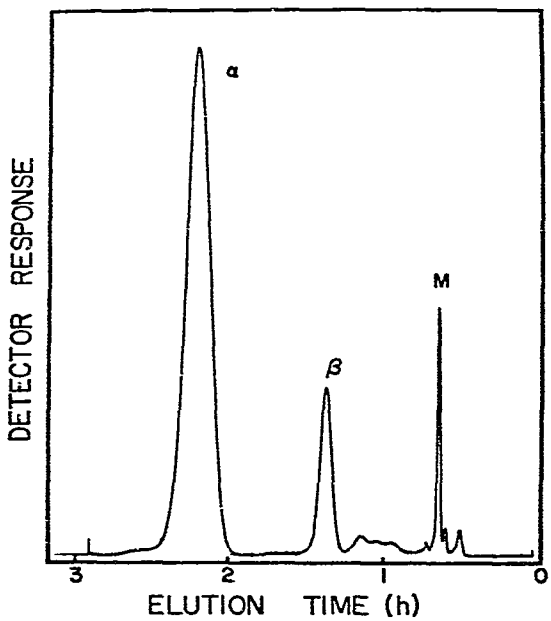

Fig. 1. Chromatographic analysis[3] of the deacetylated, Helferich-reaction products. [Peaks: M, mannose; α, *p*-nitrophenyl α-D-mannopyranoside; β, *p*-nitrophenyl β-D-mannopyranoside.]

solution was analyzed[3] to determine the content of **2** and **3**; the yields of **2** and **3** were, reproducibly, 8–10% and 61–65%, respectively. A typical chromatogram is shown in Fig. 1. Upon concentration of the decationized, deacetylation mixture, crystalline **3** was obtained in 48–52% yield. The aqueous mother liquor (20–50 ml),

*Less than a minute was required for the reactants to melt enough to allow efficient stirring. Efficient stirring was important for satisfactory yields of products.

which was now enriched in **2**, was applied to a column (5 × 80 cm) of Dowex 50 X-4 (Na^+), water-jacketed at 52°, and eluted with water[4]. Usually, several batches of the mother liquor just described were combined for one chromatographic treatment. An example of an elution profile is shown in Fig. 2. Fractions of peaks containing **2** and **3** were separately combined, and concentrated to yield crystalline products: 7–8% of **2**, and 13–14% of **3** (total of 61–65%).

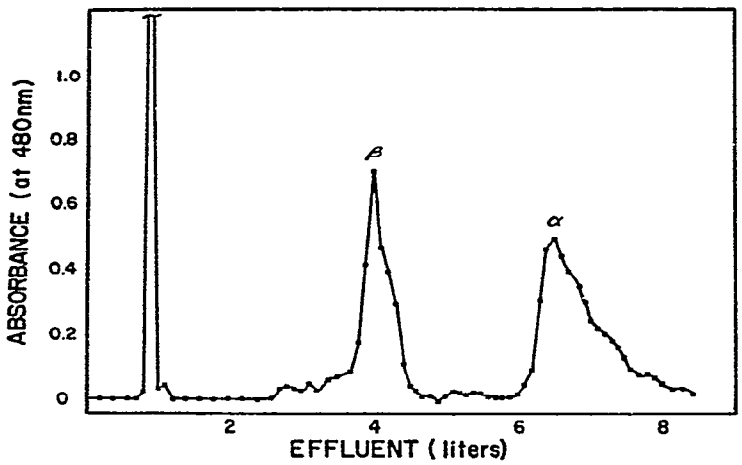

Fig. 2. Preparative, chromatographic separation[4] of *p*-nitrophenyl α,β-D-mannopyranosides [Peaks: α, *p*-nitrophenyl α-D-mannopyranoside; β, *p*-nitrophenyl β-D-mannopyranoside.]

Characterization of **2** *and* **3**. — Compound **2** was recrystallized from 95% ethanol; m.p. 206–207°, $[\alpha]_D^{20}$ −125° (*c* 0.46, water); p.m.r. data (Me_2SO-d_6): δ ~4.2 (m, 6 H, sugar-ring protons), 5.94 (d, 1 H, anomeric proton, $J_{1,2}$ <1 Hz), 7.84 (d, 2 H, aromatic-ring protons), and 8.89 (d, 2 H, aromatic-ring protons). In addition, **2** isolated by this procedure was chromatographically indistinguishable from an authentic specimen.

Compound **3**, crystallized from the column eluant, was characterized as follows: m.p. 182–184° [lit. 174° (ref. 5), 181° (ref. 6), and 183–184° (ref. 7)], $[\alpha]_D^{20}$ +155° (*c* 0.44, water) [lit. +144.5° (ref. 5), 161° (ref. 6), 145° (ref. 7)]; p.m.r. data (Me_2SO-d_6): δ 4.24 (m, 6 H, sugar-ring protons), 6.26 (d, 1 H, anomeric proton, $J_{1,2}$ 1.5 Hz), 7.98 (d, 2 H, aromatic-ring protons), and 8.90 (d, 2 H, aromatic-ring protons). In addition, **3** isolated by this procedure was chromatographically indistinguishable from an authentic specimen.

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REFERENCES

1 Y. T. Li and Y. C. Lee, *J. Biol. Chem.*, 247 (1972) 3677–3683; T. Sukeno, A. L. Tarentino, T. H. Plummer, Jr., and F. Maley, *Methods Enzymol.*, 28B (1972) 777–782.
2 B. Helferich and E. Schmitz-Hillebrecht, *Ber.*, 66 (1933) 378–383.
3 J. Schneider and Y. C. Lee, *Carbohydr. Res.*, 30 (1973) 405–408.
4 J. Schneider, H. H. Liu, and Y. C. Lee, *Carbohydr. Res.*, 39 (1975) 156–159.
5 M. A. Jermyn, *Aust. J. Chem.*, 8 (1955) 403–408.
6 O. Westphal and H. Feier, *Chem. Ber.*, 89 (1956) 582–588.
7 J. Conchie and G. A. Levvy, *Methods Carbohydr. Chem.*, 2 (1963) 345–347.