Note

The synthesis of D-mannose 6-sulphate

P. F. LLOYD AND C. H. STUART*
Department of Chemistry, University College of North Wales, Bangor, Caernarvonshire (Great Britain)

(Received October 8th, 1968)

D-Mannose 6-sulphate was required for specificity studies on sulphatases from *Patella vulgata*[1], and, although preparations of methyl α-D-mannopyranoside monosulphate[2] and of other undefined mono- and di-sulphates of D-mannose[3] have been reported, a definitive synthesis of the 6-ester had not been previously described.

Sulphation of 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose[4] with the pyridine–sulphur trioxide complex[5] in pyridine solution, followed by immediate de-*O*-acetylation with methanolic ammonia, gave crude D-mannose 6-sulphate. Electrophoretic examination indicated that it contained predominantly monosulphate, together with a little D-mannose. Purification of the product by chromatography on a cellulose column, as described[6] for D-glucose sulphates, gave two distinct fractions (Fig. 1).

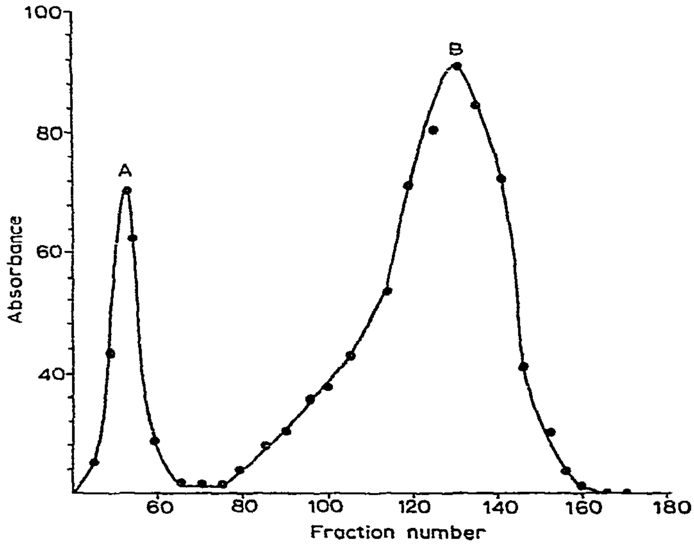

Fig. 1. Purification of D-mannose 6-sulphate on a cellulose column.

---

*Present address: Department of Biochemistry, University College, Cardiff.

The second fraction (*B*) contained only D-mannose 6-sulphate; this was unresolved by a variety of chromatographic and electrophoretic analyses, and, after isolation in the usual manner, was also analytically pure. The first fraction (*A*) contained D-mannose and D-mannose monosulphate, in approximately equivalent amounts, which may have separated in this manner as the result of complex formation.

EXPERIMENTAL

D-*Mannose 6-(barium sulphate)*. — 1,2,3,4-Tetra-*O*-acetyl-*β*-D-mannose (20.8 g; m.p. 134°; lit.[4], m.p. 135.5–136.5°) in anhydrous pyridine (80 ml) was shaken with pyridine–sulphur trioxide[5] (9.6 g) until dissolution was complete, and the mixture was kept overnight at room temperature. The products were dissolved in water (200 ml) and saturated, aqueous barium hydroxide was added to raise the pH to 7.5. The resulting suspension was concentrated *in vacuo*, and, after addition of water (240 ml), the pH was raised from 3.5 to 7 by the addition of more barium hydroxide. This procedure was repeated four times more, until the product, on addition of water, had pH 7. The insoluble, inorganic residues were removed by centrifugation, and the solution was decolorised with charcoal. On evaporation, a syrup was obtained which crystallised in part when pentyl alcohol was added to its solution in methanol; m.p. 169° (dec.). Recrystallisation caused substantial losses and gave products having variable decomposition points (156–169°). The syrupy product was therefore deacetylated, without purification, by dissolution in dry methanol (260 ml) presaturated at 0° with ammonia, for 12 h. The solution was then evaporated, and the residue, after being washed with alcohol (3 × 400 ml), was dissolved in water (160 ml), and the solution was decolorised with charcoal and concentrated to 15 ml. On addition of methanol (300 ml), the crude product was precipitated, washed with dry ether, and dried over P_2O_5; yield, 11.1 g.

The crude product (6.8 g) in water (12 ml) was applied to a column (80 × 6 cm) of standard-grade cellulose (Whatman) and eluted with 80% aqueous ethanol containing 0.33% formic acid. The fractions (50 ml) were analysed for carbohydrate content[7], appropriately combined (peak *A*, 48–65; peak *B*, 82–160), and concentrated to 12 ml, and methanol (240 ml) and ether (100 ml) were added. The product (4.2 g, 30%) from peak *B*, dried over P_2O_5, had $[\alpha]_D^{20}$ +15.2° (*c* 0.5, water) [Found: Ba, 20.9; S, 9.6. $(C_6H_{11}O_9S)_2Ba$ calc.: Ba, 20.9; S, 9.8%].

ACKNOWLEDGMENTS

We thank Professor S. Peat, F. R. S., for his interest in this work, and the Science Research Council for financial assistance.

REFERENCES

1 P. F. Lloyd and R. J. Fielder, *Biochem. J.*, 105 (1967) 33P.
2 R. B. Duff and E. G. V. Percival, *J. Chem. Soc.*, (1941) 830.

3 F. Egami, *J. Chem. Soc. Japan*, 59 (1938) 1034; 61 (1940) 592; 63 (1942) 763.
4 D. D. Reynolds and W. L. Evans, *J. Amer. Chem. Soc.*, 62 (1940) 66.
5 P. Baumgarten, *Ber.*, 59 (1926) 1166; R. B. Duff, *J. Chem. Soc.*, (1949) 1597.
6 J. R. Turvey, M. J. Clancy, and T. P. Williams, *J. Chem. Soc.*, (1960) 926.
7 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350.