Carbohydrate Research, 32 (1974) 31–36

THE CHEMICAL SYNTHESIS OF POLYSACCHARIDES

PART III. SYNTHESIS OF 6-*O*-α-D-MANNOPYRANOSYL-D-MANNOSE AND 4-*O*-α-D-MANNOPYRANOSYL-D-MANNOSE. ISOLATION OF A (1→6)-LINKED D-MANNAN

EUGENE O'BRIEN, ELIZABETH E. LEE, PROINNSIAS S. O'COLLA, AND URSULA EGAN
Chemistry Department, University College, Galway (Ireland)
(Received May 31st, 1973; accepted for publication, August 10th, 1973)

ABSTRACT

When 1,2,3,4-tetra-*O*-acetyl-α-D-mannopyranose was fused with a catalytic amount of toluene-*p*-sulphonic acid, 6-*O*-α-D-mannopyranosyl-D-mannose and 4-*O*-α-D-mannopyranosyl-D-mannose were isolated after deacetylation of the reaction mixture. No β-D-linked disaccharide was detected in the reaction mixture. When the corresponding β-D-tetra-acetate was fused with zinc chloride as catalyst, higher oligomers were formed, and a D-mannan was isolated and shown to be mainly an α-(1→6)-linked polymer having d.p. of 10. With 5% of zinc chloride, the α-D-tetra-acetate showed oligosaccharide formation, and yielded a smaller proportion of a (1→6)-linked D-mannan.

INTRODUCTION

In earlier papers[1,2], we described a method for the melt-polymerisation of partially acetylated D-glucose derivatives which has opened up a new route towards the synthesis of specific D-glucose oligosaccharides incorporating β or both β and α linkages. We now report on the application of the technique to the polymerisation of α(and β)-D-mannose 1,2,3,4-tetra-acetates.

RESULTS AND DISCUSSION

In preliminary experiments, paper-chromatographic analysis showed that toluene-*p*-sulphonic acid was an effective catalyst in the formation of oligosaccharides by polymerisation of 1,2,3,4-tetra-*O*-acetyl-α-D-mannopyranose. It was then possible to determine the proportion of catalyst, temperature, and heating period required for the formation of optimum yields of disaccharides. Amounts of toluene-*p*-sulphonic acid in excess of 0.25% caused charring, and reaction temperatures in excess of 100° were required for the necessary fusion of the acetate. Similar experiments with the β-D-tetra-acetate gave no significant indication of polymerisation.

In a large-scale experiment, the α-D-tetra-acetate (14 g) was heated with 0.25% (35 mg) of toluene-*p*-sulphonic acid for 40 min at 110–120°/20 mmHg. After dea-

cetylation, the product mixture was found to contain mannose, disaccharides, and higher oligomers. Two disaccharides (*A* and *B*) contaminated with mannose were isolated by carbon-column chromatography and purified by paper chromatography. The physical constants of disaccharide *B* (1.7 g) and of the product obtained on treatment with acetic anhydride–sodium acetate were in reasonable agreement with the literature data for 6-*O*-α-D-mannopyranosyl-D-mannopyranose[3], and its β-octa-acetate[4], respectively.

Disaccharide *A* (500 mg), which yielded a crystalline octa-acetate, was characterised as 4-*O*-α-D-mannopyranosyl-D-mannose in the following way. On hydrolysis with 0.5M sulphuric acid, *A* gave mannose only. Methylation analysis showed that *A* was (1→4)-linked, as g.l.c. of the derived methyl glycosides revealed equimolar amounts of 2,3,6-tri-*O*-methyl and 2,3,4,6-tetra-*O*-methyl derivatives of mannose. There is reported no direct synthesis of 4-*O*-α-D-mannopyranosyl-D-mannose, although it has been obtained[5], *inter alia*, from the partial acetolysate of ivory nut (*Phytelephas macrocarpa*) mannan. The formation of an α-(1→4)-linkage may be rationalized on the basis that migration of acetyl groups can be partially effected under acidic reaction conditions. Molecular models show the possibility of a 4→6 acetyl shift as a prelude to the formation of α-(1→4)-linked D-mannopyranose residues. The prediction of the α-D configuration of the (1→4)-linked disaccharide is in accordance with the probable reaction mechanism[2].

When 1,2,3,4-tetra-*O*-acetyl-α-D-mannopyranose, used in the above experiments and which was homogeneous in t.l.c., was methylated with diazomethane–boron trifluoride etherate[6] and the product deacetylated, 6-*O*-methyl-D-mannose was the only sugar isolated, and a convenient synthesis is thereby provided. This methyl sugar has recently been prepared[7], in good yield, *via* the reaction of diazomethane–boron trifluoride etherate with methyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside.

Methylation of 1,2,3,4-tetra-*O*-acetyl-α-D-mannopyranose with Purdie's reagents was sluggish and was preceded by acetyl migration, as 4-*O*-methyl-D-mannose (much) and D-mannose were detected after deacetylation of the reaction products. Similar acetyl migrations under mildly alkaline conditions are well known[8]. Methylation with diazomethane–boron trifluoride etherate of the tetra-acetate, isolated by solvent extraction of the Purdie methylation product, yielded 3-*O*- and 4-*O*-methyl-D-mannose (major components) together with 2-*O*-methyl-D-mannose, D-mannose, and a trace of 6-*O*-methyl-D-mannose.

Chromatography indicated that good yields of oligomers were obtained from both the α and β anomers of 1,2,3,4-tetra-*O*-acetyl-D-mannopyranose, using zinc chloride as catalyst in melt polymerisation. Two polymannoses, *P1* {5.6%, $[\alpha]_D$ +69° (water)} and *P2* {5%, $[\alpha]_D$ +71° (water)} were thus obtained from the β and α anomers, respectively, after polymerisation (at 120–140° for 20–25 min), deacetylation, and dialysis.

P1 and *P2* contained no chromatographically mobile sugars, and each yielded mannose on acid hydrolysis. *P1* gave no reaction with concanavalin A, indicating that it was a linear polymer[9]. The specific rotations, by comparison with the values

(+47 to +95°) for a variety of natural α-D-linked, branched mannans[10], and those (+55 to +90°) for (1→6)-linked mannans[11] prepared by polymerisation of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-mannopyranose, indicated a preponderance of α-D linkages in *P1* and *P2*. Methylation analysis of *P1* and *P2* revealed the presence of mainly (1→6)-linked D-mannopyranose residues. Methyl 2,3,6-tri-*O*-methyl-D-mannopyranoside was a minor product in the methylation analysis, and the ratio of tri- to tetra-*O*-methyl-D-mannose was ~1:8.

Thus, we have shown that the glucose polymerisation technique[1,2] may be duplicated with 1,2,3,4-tetra-*O*-acetyl-α- and β-D-mannopyranose. In accordance with the probable reaction mechanisms[2], the 1,2-*trans* tetra-acetate in the presence of toluene-*p*-sulphonic acid gave α-D-linked oligomers, and fusion of either anomer with zinc chloride as catalyst gave mainly α-(1→6)-linked D-mannans.

EXPERIMENTAL

Solutions of sugars were concentrated *in vacuo* at temperatures below 40°. Optical rotations were determined with a Bellingham and Stanley Polarimeter Model B. Chromatography on Whatman No. 1 or 3MM paper was effected with the following solvent systems: *A*, butyl alcohol–pyridine–water (6:4:3); *B*, butyl alcohol–pyridine–water–benzene (5:3:3:1, upper layer); *C*, butyl alcohol–acetic acid–water (4:1:5, upper layer); *D*, butyl alcohol–ethanol–water (5:1:4, upper layer). Paper electrophoresis was carried out in borate buffer (pH 10) at ~40 volts/cm. Sugar acetate mixtures were deacetylated with sodium methoxide in methanol–chloroform. Sugars were fractionated on Hopkins and Williams charcoal and Celite (1:1) by stepwise elution with water and aqueous ethanol. G.l.c. was carried out on a Pye–Argon gas chromatograph at nitrogen flow-rates of ~100 ml/min on columns of PEGA on Celite at 175°, for methyl sugars. Retention times (*T*) are given relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside. Ascending t.l.c. was performed on plates coated with Merck Silica Gel F-254, using benzene containing 4% of methanol. Free sugars were detected with aniline hydrogen phthalate, and sugar acetates with the ferric hydroxamate reagent. The polymerisation procedure is described in Part II of this series[2].

1,2,3,4-Tetra-O-*acetyl-6*-O-*methyl*-α-D-*mannopyranose* (**1**). — A solution of syrupy 1,2,3,4-tetra-*O*-acetyl-α-D-mannopyranose[12] (260 mg, homogeneous in t.l.c.) in dichloromethane (5 ml) was cooled to 0°. Boron trifluoride etherate (0.01 ml) was added and the solution was kept at 0° during the addition of diazomethane in dichloromethane at 0°, polymethylene was filtered off, and the filtrate evaporated to give **1** as a syrup, which showed only one component on t.l.c., had $[\alpha]_D$ +160° (*c* 0.18, chloroform), and showed no i.r. absorption for hydroxyl.

Anal. Calc. for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.07. Found: C, 49.96; H, 5.96.

6-O-*Methyl*-D-*mannose*. — To a solution of **1** (200 mg) in methanol (10 ml) was added 0.1M methanolic sodium methoxide (1 ml) and the solution was kept overnight at room temperature. Cations were removed from the solution with Amberlite IR-120(H^+) resin which was then evaporated to give the title compound

as a chromatographically homogeneous (detection with aniline hydrogen phthalate) syrup (100 mg), $[\alpha]_D$ +14° (*c* 0.2, chloroform), R_{Man} 1.4 (solvent *D*), M_G 0.58; lit.[13] $[\alpha]_D$ +15.3° (chloroform).

*Polymerisation of 1,2,3,4-tetra-*O*-acetyl-α-*D*-mannopyranose.* — A solution of **1** (14 g), in acetone containing toluene-*p*-sulphonic acid (0.1%, 35 ml), was evaporated under diminished pressure at room temperature and the residue was heated foı 40 min at 120°/20 mmHg. A solution of the deep yellow, translucent melt in chloroform (60 ml) was treated with 0.5M methanolic sodium methoxide (60 ml). After 8 h at room temperature, the free sugars were extracted with water (150 ml), and the extract was acidified with acetic acid and concentrated. Chromatography of the residue revealed mannose, di- and tri-saccharides, and traces of higher oligosaccharides. The sugars were fractionated on a column (40 × 720 mm) of charcoal–Celite. Fractions (500 ml) were collected and concentrated, and the residues were examined by p.c. (solvents *A* and *B*).

Fractions 2–4, eluted with water, contained salts; fractions 6–44, eluted with water, and fractions 45–49, eluted with 2.5% aqueous ethanol, contained D-mannose (3.89 g).

Fractions 50–80, eluted with 2.5% aqueous ethanol, contained a mixture of 6-*O*-α-D-mannopyranosyl-D-mannose and 4-*O*-α-D-mannopyranosyl-D-mannopyranose, contaminated with traces of mannose and the faster-moving anhydro sugar which was detected with the potassium periodate–cuprate spray[23]. The latter compound was not identified but may have been 1,6-anhydro-β-D-mannopyranose. Further fractionation on Whatman No. 3 paper (solvent *A*) gave (*a*) 6-*O*-α-D-mannopyranosyl-D-mannose (1.7 g), m.p. 188–190°, $[\alpha]_D$ +50° (*c* 0.9, water), M_G 0.66; lit.[14] m.p. 196–197°, $[\alpha]_D$ +52°, M_G 0.61. The disaccharide was characterised as the β-D-octa-acetate, m.p. 146°, $[\alpha]_D$ +55° (*c* 0.3, ethanol); lit.[4], m.p. 152–153°, $[\alpha]_D$ +19.6° (chloroform). (*b*) 4-*O*-α-D-Mannopyranosyl-D-mannose (500 mg), $[\alpha]_D$ +80° (*c* 0.6, water), with R_{Gal} 0.45 (solvent *B*), and M_G 0.71; lit.[5] $[\alpha]_D$ +49°. The disaccharide yielded a β-D-octa-acetate, m.p. 212–216°, $[\alpha]_D$ +90° (*c* 0.12, chloroform).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.55; H, 5.64. Found: C, 49.11; H, 5.58.

The disaccharide (80 mg) was methylated by two treatments with methyl sulphate and 40% aqueous sodium hydroxide. A solution of the partially methylated product in *N*,*N*-dimethylformamide was treated with methyl iodide (2.5 ml) and silver oxide (2 g) at room temperature for 18 h. T.l.c. then indicated that methylation was complete[19]. P.c. (solvent *C*) of a hydrolysate of the methylated material revealed a tri-*O*-methyl-D-mannose (R_G 0.86) and 2,3,4,6-tetra-*O*-methyl-D-mannose (R_G 0.98). G.l.c. of the derived methyl mannosides gave peaks having *T* values (1.4 and 4.6) corresponding to the values reported[15,16] for methyl 2,3,4,6-tetra-*O*-methyl-D-mannopyranoside and methyl 2,3,6-tri-*O*-methyl-D-mannopyranoside, respectively, and in a molar ratio of unity.

*Purdie methylation of 1,2,3,4-tetra-*O*-acetyl-α-*D*-mannopyranose.* — The title acetate (200 mg; R_F 0.41, t.l.c., ether) was treated with methyl iodide (3 ml) and silver oxide (150 mg), and the mixture was maintained at 40° under reflux for 5 min and

then shaken overnight at room temperature. After 14 h, t.l.c. (solvent *A*) showed >50% conversion of the starting material into the faster-moving reaction product. Chloroform was then added and the mixture was filtered. The filtrate was concentrated to a syrup which was dissolved in benzene (40 ml) and extracted with water (4 × 30 ml). Extraction of the aqueous phase with chloroform and evaporation of the extract yielded a syrup (120 mg), which was shown by t.l.c. (ether) to contain isomerized tetra-acetate (R_F 0.48), contaminated with traces of 1,2,3,4-tetra-*O*-acetyl-α-D-mannopyranose (R_F 0.41). A solution of the syrup (77 mg) in dichloromethane (5 ml) was treated with diazomethane–boron trifluoride etherate in the usual manner. Polymethylene was removed, and the filtrate was evaporated. Paper electrophoresis of a portion (~20 mg) of the resulting syrup, after deacetylation, revealed the presence of 3-*O*-methyl- (M_G 0.63) and 4-*O*-methyl-D-mannose (M_G 0.54) as major components, identified by comparison with authentic samples, together with the presumed 2-methyl ether (M_G 0.43), 6-methyl ether (M_G 0.58), and D-mannose (M_G 0.70) as trace components.

T.l.c. of the residual benzene solution showed only one component, corresponding to methylated tetra-acetate. Deacetylation of a portion (10 mg) of the syrup (~70 mg) obtained on evaporation of the benzene, followed by electrophoresis, revealed 4-*O*-methyl-D-mannose (M_G 0.54) and D-mannose (M_G 0.70). The occurrence of free sugar among the products of methylation may be the result of ortho-ester formation during the sluggish reaction of the tetra-acetate with Purdie's reagents.

Polymerisation of 1,2,3,4-tetra-O-acetyl-β-D-mannopyranose. — A mixture of 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose[17] (7.1 g, m.p. 135°) and zinc chloride (350 mg) was heated for 20 min at 140°/20 mmHg. The melt was deacetylated, and the resulting, water-soluble material was dissolved in water (70 ml). The filtered solution was dialysed against frequent changes of distilled water during 60 h. The dialysate contained a mixture (2.80 g) of mannose and a homologous series of oligosaccharides (2 → 7) which were resolved against a background of fainter spots by p.c. (solvent *A*) and multiple development[18]. The polymer solution was further dialysed against running tap-water for 30 h, concentrated to a small volume (15 ml), and treated with 6 vol. of ethanol and 1 vol. of acetone. The precipitated polysaccharide *P1* (200 mg) had $[\alpha]_D$ +69° (*c* 0.64, water) and was non-mobile in p.c. (72-h development, solvent *A*).

Methylation of polysaccharide P1. — Methylation involved two Haworth and three Purdie methylations. T.l.c. then indicated that the methylation was complete[19]. P.c. of a hydrolysate of the methylated material revealed 2,3,4,6-tetra-*O*-methyl-D-mannose (red spot, R_G 0.98; lit.[20] R_G 0.96) and a tri-*O*-methyl-D-mannose (red spot, R_G 0.84; lit.[21] 0.86 for 2,3,4-tri-*O*-methyl-D-mannose), and a trace of a di-*O*-methyl-mannose (R_G 0.59). G.l.c. of the derived methyl mannosides gave peaks having *T* values (1.4 and 2.94) corresponding to authentic methyl 2,3,4,6-tetra- and methyl 2,3,4-tri-*O*-methyl-D-mannoside, respectively, and in the ratio ~1:8. A small peak (*T* 4.56) corresponding[16] to methyl 2,3,6-tri-*O*-methyl-D-mannopyranoside was also detected.

A hydrolysate of the material was separated on sheets of Whatman No. 1 paper. The tri-*O*-methyl fraction (6.5 mg) was oxidised (bromine water) and the lactones were isolated (partly crystalline). P.c. (butyl alcohol–ethanol–water, 3:1:1) revealed 2,3,4-tri-*O*-methyl-D-mannonolactone (R_F 0.77; lit.[22] 0.75) and also (tentative identification) 2,3,6-tri-*O*-methyl-D-mannonolactone (R_F 0.67, hydroxamine acid spray).

Polymerisation of 1,2,3,4-tetra-O-acetyl-α-D-mannopyranose. — The tetraacetate (3.93 g) was heated with zinc chloride (200 mg) for 25 min at 120°/20 mmHg. The deacetylated reaction product was dialysed against tap water for 30 h, and polysaccharide *P2* (100 mg), $[\alpha]_D$ +71° (*c* 0.48, water), was isolated as described for *P1*. It was non-mobile in p.c. (solvent *A*). The dialysate contained mannose and an homologous series of sugars against a background of fainter spots (p.c., multiple development, solvent *A*). The polymer was not further examined.

ACKNOWLEDGMENT

The authors thank the National Science Council of Ireland for a grant and for a scholarship (E.O'B.).

REFERENCES

1 D. McGrath, E. E. Lee, and P. S. O'Colla, *Carbohyd. Res.*, 11 (1969) 453.
2 D. McGrath, E. E. Lee, and P. S. O'Colla, *Carbohyd. Res.*, 11 (1969) 461.
3 P. A. J. Gorin and A. S. Perlin, *Can. J. Chem.*, 37 (1959) 1930.
4 E. A. Talley, D. D. Reynolds, and W. L. Evans, *J. Amer. Chem. Soc.*, 65 (1943) 575.
5 G. O. Aspinall, R. B. Rashbrook, and G. Kessler, *J. Chem. Soc.*, (1958) 215.
6 I. O. Mastronardi, S. M. Flematti, J. O. Deferrari, and E. G. Gros, *Carbohyd. Res.*, 3 (1966) 177.
7 E. G. Gros and E. M. Gruneiro, *Carbohyd. Res.*, 14 (1970) 409.
8 W. A. Bonner, *J. Org. Chem.*, 24 (1959) 1388.
9 R. Robinson and I. J. Goldstein, *Carbohyd. Res.*, 13 (1970) 425.
10 P. A. J. Gorin and A. S. Perlin, *Can. J. Chem.*, 34 (1956) 1796.
11 F. Frechet and C. Schuerch, *J. Amer. Chem. Soc.*, 91 (1969) 1161.
12 D. D. Reynolds and W. L. Evans, *J. Amer. Chem. Soc.*, 62 (1940) 66.
13 N. Handa and R. Montgomery, *Carbohyd. Res.*, 11 (1969) 467.
14 S. Peat, J. R. Turvey, and D. Doyle, *J. Chem. Soc.*, (1961) 3918.
15 G. O. Aspinall, *J. Chem. Soc.*, (1963) 1676.
16 D. S. Geddes and K. C. B. Wilkie, *Carbohyd. Res.*, 23 (1972) 349.
17 B. Helferich and J. F. Leete, *Ber.*, 62 (1929) 1552.
18 A. Jeanes, C. S. Wise, and R. J. Dimler, *Anal. Chem.*, 23 (1951) 415.
19 A. M. Unrau and J. M. Choy, *Carbohyd. Res.*, 14 (1970) 151.
20 E. L. Hirst and J. K. N. Jones, *Discussions Faraday Soc.*, 7 (1949) 268.
21 C. M. Rafique and F. Smith, *J. Amer. Chem. Soc.*, 72 (1950) 4634.
22 J. K. N. Jones and W. H. Nicholson, *J. Chem. Soc.*, (1958) 29.
23 T. G. Bonner, *Chem. Ind. (London)*, (1960) 345.