1277. *Aminosaccharides. Part II.*[1] *Synthesis of* 6-O-(2-*Amino*-2-*deoxy*-α-D-*glucopyranosyl*)-D-*galactose Hydrochloride and* 6-O-(2-*Amino*-2-*deoxy*-β-D-*glucopyranosyl*)-D-*galactose Hydrochloride and Derivatives* [2]

By P. F. Lloyd and G. P. Roberts

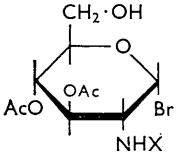
Condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide (I) with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (II) in chloroform in the presence of pyridine gave a mixture of 6-*O*-[2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (III) (30% yield) and 6-*O*-[2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (IV) (15% yield). The latter (IV) was the main product (29% yield) when the condensation was effected in nitromethane in the presence of silver carbonate.

Earlier studies [1,3,4] established that condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucosyl bromide (I) with simple alcohols usually furnishes a mixture of both α- and β-*O*-glycosides, the proportions of which are dependent on the solvent and catalyst employed. Thus condensation of the bromo-compound (I) with alcohols in polar solvents in the presence of silver carbonate furnished high yields of β-*O*-glycosides, and the products were predominantly α-*O*-glycosides when pyridine was used as catalyst and the

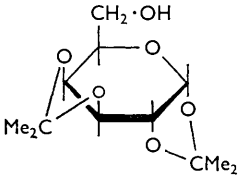
[1] Part I, P. F. Lloyd and G. P. Roberts, *J.*, 1963, 2962.
[2] Preliminary communication, *Proc. Chem. Soc.*, 1960, 250.
[3] P. F. Lloyd and M. Stacey, *Chem. and Ind.*, 1955, 917.
[4] P. F. Lloyd and M. Stacey, *Tetrahedron*, 1960, **9**, 116.

reaction conducted in non-polar solvents. This Paper reports the extension of these reactions to the synthesis of disaccharides with α- and β-bioside linkages.

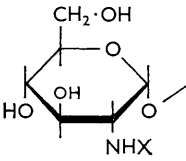
The bromo-compound (I) reacted with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (II) (10 moles) in chloroform containing pyridine, conditions which, on the basis of model experiments [1] in which ethanol was the aglycon alcohol, were expected to yield approximately equal amounts of α- and β-biosidically-linked disaccharides. The products of



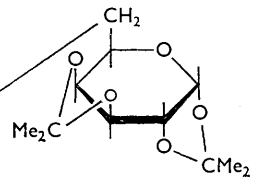
I) X = 2,4-$(NO_2)_2C_6H_3$



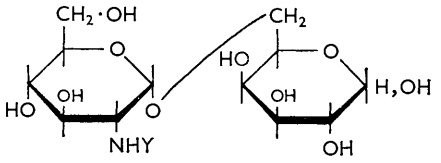
(II)



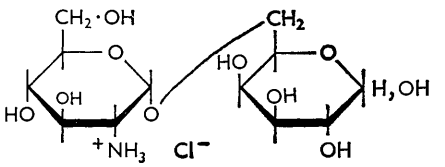
(III)



(IV) as for (III) but with β-bioside linkage



(V) Y = 2,4-$(NO_2)_2C_6H_3$
(VII) Y = Ac
(VIII) As for (V) but with β-bioside linkage
(X) As for (VII) but with β-bioside linkage



(VI)
(IX) as for (VI) but with β-bioside linkage

reaction were analysed chromatographically on a column containing Magnesol and Celite, which resulted in the separation of the disaccharide fraction from the slower moving 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)α-D-glucose. De-*O*-acetylation of the disaccharide fraction with methanolic ammonia followed by a second chromatographic analysis on a Magnesol–Celite column removed further trace amounts of impurities. Electrophoresis and paper chromatography of the product established that two components were present, and application of a modification of the method of Anison, James, and Morgan,[5] involving borate-complex formation and partition chromatography led to their complete resolution. The first band to be eluted was the α-linked disaccharide (III) (30% yield), followed by the disaccharide containing the β-linkage (IV) (15% yield). The structures allocated were based initially on optical rotational measurements.

In order to confirm the structures provisionally assigned, a second condensation of the bromo-compound (I) and the galactose derivative (II) was carried out in nitromethane in the presence of silver carbonate and anhydrous calcium sulphate. Purification of the condensation product, effected by column-chromatography of the initial and then subsequently of the de-*O*-acetylated product, gave a disaccharide (29% yield) which, from the method of condensation employed,[1] was expected to contain the β-linkage. It was identical with the disaccharide derivative obtained in 15% yield in the first condensation.

Treatment of the α-linked disaccharide (III) with hot 0·1N-sulphuric acid effected smoothly the removal of the isopropylidene residues, furnishing the *N*-2,4-dinitrophenyl derivative of the aminodisaccharide (V). Removal of the *N*-dinitrophenyl group from (III), by alkaline hydrolysis, followed by removal of the isopropylidene groups, by acid hydrolysis, gave the parent aminodisaccharide, which was isolated as its hydrochloride (VI). *N*-Acetylation of the latter gave the *N*-acetylated α-linked disaccharide (VII).

By a similar reaction sequence, the β-linked disaccharide (IV) was converted into the *N*-dinitrophenyl disaccharide (VIII), the aminodisaccharide hydrochloride (IX), and the

[5] E. F. Anison, A. T. James, and W. T. J. Morgan, *Biochem. J.*, 1951, **48**, 477.

N-acetylated disaccharide (X). 6-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose (X) was prepared previously by a different route.[6]

Experimental

The solvents were purified by methods previously described.[1] All condensations were carried out under rigorously anhydrous conditions. Evaporation and distillation were carried out under reduced pressure unless otherwise stated. The Magnesol–Celite chromatography columns were prepared by the method described earlier.[1]

3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitroanilino*)-α-D-*glucopyranosyl Bromide* (I).—This, prepared by the method already described,[4] had m. p. 162°, $[\alpha]_D^{18}$ +46·9° (*c* 1·3 in $CHCl_3$) {lit.,[4] m. p. 160—162°, $[\alpha]_D^{19}$ +45·6° (in $CHCl_3$)}.

1,2:3,4-*Di*-O-*isopropylidene*-α-D-*galactopyranose* (II).—This, after preparation by the method of Raymond and Schroeder [7] and distillation under a high vacuum, had $[\alpha]_D^{18}$ −61·7° (*c* 0·8 in benzene).

Condensation of the Bromide (I) *with* 1,2:3,4-*Di*-O-*isopropylidene*-α-D-*galactopyranose*.—(a) *In the presence of pyridine.* The bromo-compound (I) (4·23 g.), 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (II) (20·5 g., 10 mol.), and pyridine (1·27 ml., 2 mol.) were dissolved in chloroform (250 ml.), and the solution was maintained at 50° for 100 hr., evaporated, and the dark remaining orange oil stirred with benzene. A yellow crystalline substance, insoluble in benzene, was filtered off; it was 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosylpyridinium bromide,[1] m. p. 162° (decomp.).

The benzene solution was applied to a column of Magnesol and Celite (5 : 1 w/w) (50 × 5 cm.). Elution with benzene–acetone (50 : 1) formed two yellow bands, the slower of which was chromatographically identical with 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranose.[1] The faster band was eluted from the column and re-chromatographed on a second Magnesol–Celite column but was not further resolved; the solution from this band was evaporated, and the residue deacetylated by dissolution in methanol saturated at 0° with ammonia and keeping overnight at room temperature. The deacetylated material, isolated by evaporation, was chromatographed on a column of Magnesol–Celite (5 : 1) (35 × 6 cm.). Two bands were eluted from the column with benzene–acetone (10 : 1) (3·6 l.), and a third, yellow band with benzene–acetone (1 : 2) (1·2 l.). The latter band furnished an orange solid (2·1 g.), the disaccharide nature of which was demonstrated by the presence, in acid-hydrolysates, of 2-deoxy-2-(2,4-dinitroanilino)-glucose and -galactose; the sugars were identified by paper chromatography using butanol–ethanol–water (3 : 1 : 1). Electrophoresis (0·1M-borate buffer, pH 10·0) established that two components were present. They were separated by partition chromatography as follows.

A column (47 × 6 cm.) was dry-packed by ramming down in small amounts Celite (320 g.) which had been mixed with the aqueous phase (290 ml.) of a mixture of cyclohexane–propan-1-ol–0·20M-potassium borate (pH 9·8) (5 : 1 : 1 by vol.), and washed with the organic phase of this solvent system. The orange solid (1·24 g.), dissolved in the organic phase (250 ml.), was applied to the column and eluted with further amounts of the same solvent at 2°. Two bands separated and were eluted from the column; these two fractions were dried ($MgSO_4$), and on evaporation yielded the α-linked disaccharide (III) (0·79 g., 30%) (first band) and β-linked disaccharide (IV) (0·40 g., 15%). These products were electrophoretically homogeneous and substantially pure. Trace amounts of borate were eliminated by recrystallisation from benzene–light petroleum, and also by salting-out with ammonium sulphate the disaccharides from aqueous solutions. 6-O-[2-*deoxy*-2-(2,4-*dinitroanilino*)-α-D-*glucopyranosyl*]-1,2:3,4-*di*-O-*isopropylidene*-α-D-*galactopyranose* (III) thus obtained had indefinite m. p. (softening at 80°), $[\alpha]_D^{18}$ −39·8° (*c* 0·4 in $CHCl_3$) (Found: C, 48·8; H, 5·7; N, 7·2. $C_{24}H_{33}N_3O_{14}$ requires C, 49·1; H, 5·7; N, 7·2%). The other product isolated, the β-*isomer* (IV), had m. p. 197—199°, $[\alpha]_D^{18}$ −87·9° (*c* 0·12 in $CHCl_3$) (Found: C, 49·0; H, 5·8; N, 7·2%).

(b) *In the presence of silver carbonate.* The bromo-compound (I) (2·10 g.) and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (10·23 g., 10 mol.) in nitromethane (126 ml.) were shaken with silver carbonate (10·85 g., 10 mol.) and calcium sulphate (8·0 g.) in the dark at room temperature for 142 hr. The mixture was then filtered through charcoal, evaporated

[6] R. Kuhn and W. Kirschenlohr, *Chem. Ber.*, 1954, **87**, 384.
[7] A. L. Raymond and E. F. Schroeder, *J. Amer. Chem. Soc.*, 1948, **70**, 2785.

to dryness, and the residue dissolved in benzene (30 ml.). The disaccharide component was isolated by column chromatography followed by de-*O*-acetylation and a further analysis on Magnesol–Celite as described in (a), above. Electrophoresis of the product thus obtained indicated that it was the β-disaccharide (IV) (29·4% yield) containing a trace of the α-disaccharide (III). The latter impurity was removed by recrystallisation from benzene–light petroleum. The purified product had m. p. 198—199° unchanged on admixture with the product (IV) obtained in (a) above, $[\alpha]_D^{18}$ −82·5° (*c* 0·4 in $CHCl_3$).

6-O-[2-*Deoxy*-2-(2,4-*dinitroanilino*)-α-D-*glucopyranosyl*]-D-*galactopyranose* (V) *and the Isomer* (VIII) *containing the* β-*Bioside Linkage*.—In preliminary experiments, 6-*O*-[2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (III) (3 mg.) was hydrolysed with 0·1N-, 0·5N-, and 1N-sulphuric acid (2 ml.) at 100° for 5½ hr. Aliquots were withdrawn at intervals and neutralised with lead carbonate, and the products were analysed by paper chromatography (*n*-butanol–ethanol–water, 3 : 1 : 1). Most of the isopropylidene residues were removed in 5 min. with 0·1N-sulphuric acid, and all after 0·5 hr. Only a small amount of scission of the bioside linkage occurred on heating with 0·5N-sulphuric acid for 5½ hr. or 1N-sulphuric acid for 1 hr.

The disaccharide derivative (III) (0·30 g.) was hydrolysed with 0·1N-sulphuric acid (180 ml.) at 100° for 1 hr. The solution was neutralised with barium carbonate, filtered, and evaporated to dryness, to yield an orange solid (0·27 g.). This was recrystallised by dissolution in the minimum of hot ethanol, and addition of benzene (3 vol.) and sufficient light petroleum to cause turbidity. The orange α-*disaccharide* (V) (0·21 g., 81%) thus obtained had indefinite m. p. (softening at 170°), $[\alpha]_D^{18}$ +102·8° (*c* 0·28 in MeOH) (Found: C, 42·3; H, 5·2; N, 8·0. $C_{18}H_{25}N_3O_{14}$ requires C, 42·6; H, 4·9; N, 8·3%). In a similar manner was obtained (74% yield) the β-*isomer* (VIII), m. p. 215—216°, $[\alpha]_D^{18}$ +33·3° (*c* 0·24 in H_2O) (Found: C, 42·3; H, 5·4; N, 8·5%).

6-O-(2-*Amino*-2-*deoxy*-α-D-*glucopyranosyl*)-D-*galactose Hydrochloride* (VI) *and the Isomer* (IX) *containing the* β-*Bioside Linkage*.—The disaccharide derivative (III) (0·30 g.) dissolved in acetone (24 ml.) and water (12 ml.) was shaken with ion-exchange resin Amberlite IRA-401 (OH^- form) (35 ml.) for 1 hr. until the solution had been decolourised. The mixture was filtered and the resin washed well with water–acetone. Evaporation of the filtrate and washings gave an oil which was hydrolysed with hydrochloric acid (0·1N; 23 ml.) at 100° for 1 hr. The solution was then deacidified by shaking for 45 min. with Amberlite IRA-401 (CO_3^{2-} form), and, after filtration, treated with 0·1N-hydrochloric acid until the pH was 3·5. The solution was evaporated to dryness and the product recrystallised from methanol–acetone, to yield crystals of the *hydrochloride* (VI) (0·10 g., 52%), indefinite m. p. (softening at 50—60°), $[\alpha]_D^{18}$ +112·5° (*c* 0·24 in H_2O) (Found: C, 34·8; H, 6·8; N, 3·4. $C_{12}H_{23}NO_{10},HCl,2H_2O$ requires C, 34·8; H, 6·8; N, 3·4%). In a similar manner was obtained the β-*isomer* (IX) (52% yield), m. p. 197° (decomp.), $[\alpha]_D^{18}$ +15·4° (*c* 0·28 in H_2O) (Found: C, 38·2; H, 6·7; N, 3·4. $C_{12}H_{23}NO_{10},HCl$ requires C, 38·2; H, 6·4; N, 3·7%).

6-(2-*Acetamido*-2-*deoxy*-α-D-*glucopyranosyl*)-D-*galactose* (VII).—The method employed was similar to that used [8] to prepare 2-acetamido-2-deoxy-D-glucose. The hydrochloride (VI) (0·20 g.) was dissolved in water (4 ml.), cooled at 0°, and Amberlite IRA-401 (CO_3^{2-} form) (9 ml.), methanol (0·70 ml.), water (4 ml.), and acetic anhydride (0·20 ml.) were added. The mixture was stirred for 1½ hr. at 2° and filtered. The filtrate was passed through Zeo-Karb 225 (H^+ form) (2 ml.) and then evaporated to dryness. Recrystallisation from methanol–dimethoxyethane yielded the α-*isomer* (VII) (0·13 g., 64%), m. p. indefinite (softening at 60°), $[\alpha]_D^{18}$ +125·6° (*c* 0·24 in H_2O) (Found: C, 40·5; H, 6·8; N, 3·5. $C_{14}H_{25}NO_{11},2H_2O$ requires C, 40·1; H, 6·9; N, 3·4%). The β-*isomer* (X), obtained from the hydrochloride (IX) in a manner similar to that described above (59% yield), had indefinite m. p. (softening at 50—60°), $[\alpha]_D^{18}$ +9·9° (*c* 0·10 in H_2O) [lit.,[8] $[\alpha]_D^{20}$ +9·2° (*c* 0·65 in H_2O)] (Found: N, 3·6. $C_{14}H_{25}NO_{11}$ requires N, 3·7%).

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[8] S. Roseman and J. Ludoweig, *J. Amer. Chem. Soc.*, 1954, **76**, 301.