547. *Aminosaccharides. Part I. Synthesis of α- and β-Glycosides from 3,4,6-Tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)glucosyl Bromide.*

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A method for the synthesis of α-*O*-glycosides of D-glucosamine has been developed.

Definitive syntheses of ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α- and -β-D-glucopyranoside (IV, VII) are described and condensations of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide (VIII) with alcohols have been studied. Column- and paper-chromatographic as well as electrophoretic methods for identifying and separating the reaction products have been devised. With ethanol in the presence of pyridine the bromide yields a mixture of ethyl α- and β-glycosides: the yield of the α-glycoside is highest (83%) when a large excess of the alcohol in a non-polar solvent is used. In addition to the glycosides, 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucose (IX) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosylpyridinium bromide (X) were formed with (in certain reactions) small amounts of 3,4,6-tri-*O*-acetyl-1,2-dideoxy-1,2-(2,4-dinitrophenylepimino)-α-D-glucopyranose (as XI).

Reaction of the glucosyl bromide with ethanol in the presence of silver carbonate gave mainly the β-glycoside, yields being high (83%) in nitromethane. In the presence of mercuric acetate or mercuric bromide it furnished a mixture of α- and β-glycoside in proportions depending on the solvent and the concentrations. The mechanism of the pyridine-catalysed reaction is discussed.

The derivatives of 2-deoxy-2-(2,4-dinitroanilino)-D-glucose form a coloured crystalline series of value in chemical synthesis. It has been reported[1,2] that 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide (VIII) (earlier called acetobromo-DNP-glucosamine) with benzyl alcohol in the presence of pyridine forms the benzyl α-glycoside, whereas in the presence of silver carbonate it yields the β-glycoside. This reaction appears to be general since the α- or β-methyl and α- or β-ethyl glycosides can be obtained similarly. The products were, however, difficult to isolate, and pure products were only obtainable in low yield. Consequently, Koenigs–Knorr reactions of the bromide have been investigated systematically and methods devised for the preparation and isolation in high yield of both the α- and the β-glycopyranosides. This paper reports the results.

Our studies centred mainly on reaction of the bromide (VIII) with ethanol, so the α- (IV) and β-glucoside (VII) were first synthesised definitively. The α-glycoside was obtained by *N*-dinitrophenylation and subsequent *O*-acetylation of ethyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride (II), itself prepared by catalytic removal[3] of the *N*-benzyloxycarbonyl group from ethyl 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside[4] (I) of established structure.

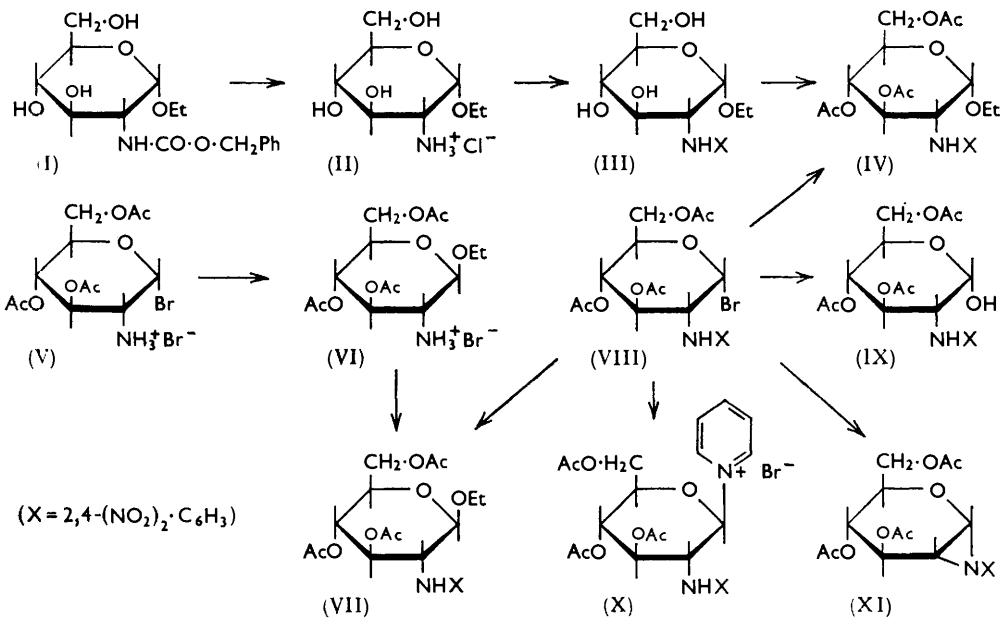
3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide[5] hydrobromide (V) was converted into the glucoside hydrobromide[6] (VI) which, on treatment with 1-fluoro-2,4-dinitrobenzene, furnished the crystalline β-glucoside (VII).

A further reference compound, 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucose (IX), which was always a by-product of condensation reactions of the bromide

[1] Lloyd and Stacey, *Chem. and Ind.*, 1955, 917.
[2] Lloyd and Stacey, *Tetrahedron*, 1960, **9**, 116.
[3] Neuberger and Pitt Rivers, *J.*, 1939, 122.
[4] Foster, Horton, and Stacey, *J.*, 1957, 81.
[5] Irvine, McNicoll, and Hynd, *J.*, 1911, **99**, 250.
[6] Irvine and Hynd, *J.*, 1913, **103**, 41.

(VIII), was prepared in 88% yield when this bromide was treated with silver carbonate and water in acetone.

Methods for the separation, identification, and estimation of the reference compounds (IV, VII, and IX) were then sought. Chromatography on columns of "Magnesol"[7] and "Celite" led to complete separation of mixtures of the three substances and their quantitative recovery; also the component present in each coloured band on the column could be identified by paper chromatography and, after deacetylation, by electrophoresis; its concentration was determined spectrophotometrically.



In the studies described below the bromide (VIII) was treated at constant temperature with ethanol in a solvent in the presence of a catalyst until reaction was complete. The products, separated as just described, were either isolated or were identified and estimated. Future application to oligosaccharide synthesis has been borne in mind and condensations which involved a limited proportion (3 mol.) of alcoholic component have been included.

Pyridine-catalysed Condensations.—Table 1 shows the results of a series of pyridine-catalysed condensations in which only the solvent was varied. In contrast to condensations

TABLE 1.

Products from the bromide (VIII) (100 mg.) and ethanol (200 mol.) in the presence of pyridine (2 mol.) in various solvents (4·0 ml.) at 50° (24 hours).

Solvent	Dielectric const. of solvent	Yields (mg.) of (IV)	(VII)	(IX)	Ratio IV/VII	Solvent	Dielectric const. of solvent	Yields (mg.) of (IV)	(VII)	(IX)	Ratio IV/VII
$MeNO_2$	39	52·5	18·1	3·8	2·9	$H{\cdot}CO{\cdot}NMe_2$	—	1·6	0·9	93·8	1·7
MeCN	37	50·0	17·5	3·9	2·9	Pyridine	12·5	0·8	0·5	2·2	1·5
EtOH	25·8	52·5	11·3	8·5	4·6	$CHCl_3$	5·1	76·3	8·1	3·8	9·4
$COMe_2$	21·4	41·9	7·5	29·1	5·6	Dioxan	2·2	64·7	12·5	4·5	5·2

conducted in solvents of high dielectric constant, those in non-polar solvents resulted in (i) a high yield of the α-glycoside (IV) and (ii) a high ratio of α- to β-glycoside. Acetone

[7] Wolfrom, Lemieux, and Olin, *J. Amer. Chem. Soc.*, 1949, **71**, 2870.

and, especially, dimethylformamide proved unsuitable. In a further series (Table 2) in dioxan or chloroform, only the proportion of the alcohol (ethanol) was varied; as the concentration of ethanol diminished so also the yield of α-glycoside and the ratio of α- to β-glycoside fell.

TABLE 2.

Products from the bromide (VIII) (100 mg.) and ethanol in the presence of pyridine (2 mol.) in dioxan or chloroform (total vol. 6 ml.) at 50° (92 hours).

Solvent	Ethanol (mol.)	Yields (mg.) of (IV)	(VII)	(IX)	Ratio, IV/VII	Solvent	Ethanol (mol	Yields (mg.) of (VII)	(IX)	Ratio, IV/VII
Dioxan	200	68·8	11·3	4·7	6·1	Chloroform	200	10·0	4·6	7·8
,,	100	50·3	22·5	5·5	2·2	,,	10	30·6	4·1	1·0
,,	50	34·1	25·6	7·5	1·3	,,	3	20·6	4·7	0·7
,,	20	17·5	21·9	8·8	0·8					
,,	10	13·5	18·8	12·5	0·7					

Chromatography on " Magnesol " led to elution of the main products (IV), (VII), and (IX) in that order but some other products were also formed in low yield. In every case an insoluble residue remained when, before chromatography, the products were stirred with benzene. That this was the pyridinium compound (X) was proved by its analysis, solubility in water, neutrality to litmus, and feeble reduction of Tollens's and Fehling's solutions, its possession of a dinitrophenyl group and bromide ion, behaviour on paper electrophoresis (coloured cation), liberation of pyridine when it was heated with alkali, and formation in high yield when the bromide (VIII) reacted with pyridine. Other workers [8] isolated similar compounds on reaction of glycosyl halides with pyridine.

When the products of Koenigs–Knorr condensation in dioxan or acetone were analysed on a " Magnesol " column the first band eluted contained, not the α-glycoside (IV), but a new substance (P) in low yield (1—3%). This appears to be 3,4,6-tri-*O*-acetyl-1,2-dideoxy-1,2-(*N*-2,4-dinitrophenylepimino)-α-D-glucopyranose (XI). The evidence is as follows. On a paper chromatogram, it moved at the same rate as the β-glycoside (VII) but after deacetylation it migrated electrophoretically in borate at a rate equivalent to that of the deacetylated α-glycoside (IV). It formed yellow crystals and its ultraviolet absorption spectrum, which included a band at 340 mμ, confirmed its containing an *N*-2,4-dinitrophenyl group. Although this compound was non-reducing it was rapidly converted, on treatment with cold dilute acetic acid, into the reducing triacetate (IX), so that it was neither a disaccharide nor an *O*- or *N*-glycosyl derivative. It was saturated (*i.e.*, not a glycoseen). It contained *O*-acetyl groups which were removed completely on treatment with methanolic ammonia, giving a yellow solid, m. p. 171° which, in dilute acid solution, was rapidly converted into *N*-2′,4′-dinitrophenylglucosamine (chromatographic evidence); presumably the imino-group remained intact during de-esterification. It has been postulated [9] that an intermediate imino-compound is formed during the base-catalysed conversion of 2-deoxy-2-toluene-*p*-sulphonamido-β-D-glucosyl fluoride into β-glycosides and 1,6-anhydride.

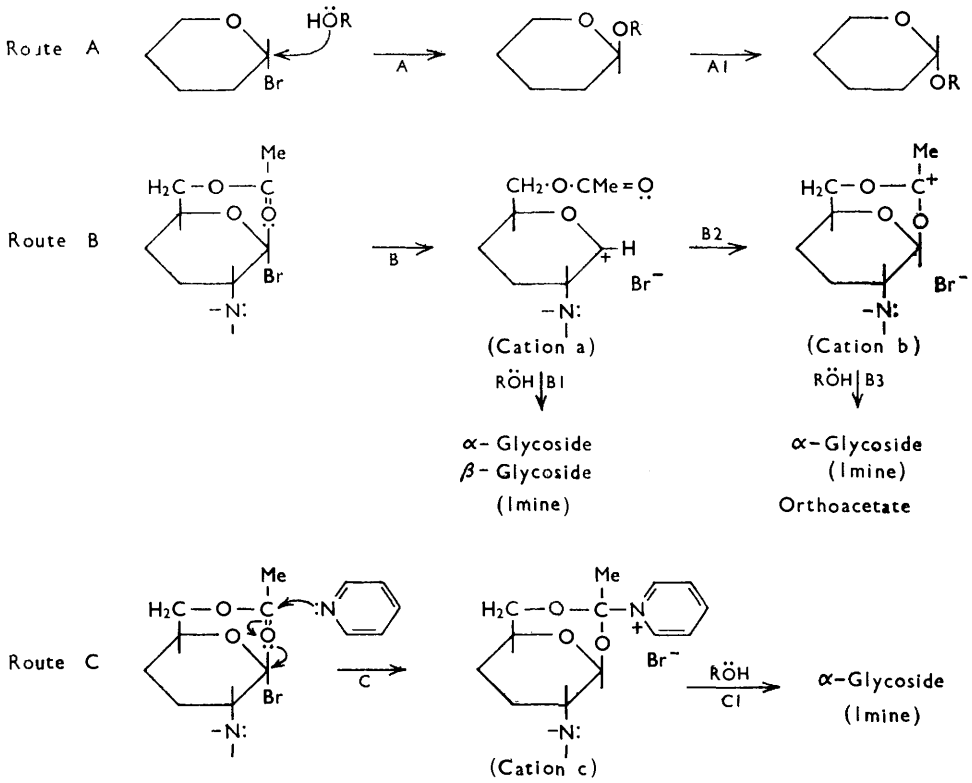
A further product (Q) was often present in traces when moderately low proportions (<100 mol.) of ethanol were used in the condensation. This compound was observed as a separate faint yellow band on the column and was eluted after the α- (IV) but before the β-glycoside (VII). It was photosensitive and, although initially it was yellow with an absorption spectrum similar to that of the glycoside (VII), exposure to daylight or violet light converted it into a white substance (R) which was insoluble in benzene an a different spectrum (no absorption band at 340 mμ). The identities of compou and R are not known. Occasionally compound Q was incompletely separated from t β-glycoside (VII); the amount of the latter present could, however, still be estimate

[8] Fischer and Raske, *Ber.*, 1910, **43**, 1750; Micheel and Köchling, *Chem. Ber.*, 1959, **92**, 2832.
[9] Micheel and Wulff, *Chem. Ber.*, 1956, **89**, 1521; Micheel and Michaelis, *ibid.*, 1958, **91**, 188.

provided the solution was kept in daylight until the value of its optical density at 340 mμ had become constant (several days).

Mechanism of the Pyridine-catalysed Condensations.—Possible mechanism for Koenigs–Knorr condensations have been discussed by a number of workers.[10] The course of reaction may be influenced by participation of neighbouring groups [11] and by the nature of the solvents.[12,13] Replacements which involve glycosyl halides may proceed by S_N1 [14] or S_N2 mechanisms.[10,13,15]

The bromide (VIII), like similar bromides, almost certainly has the α-configuration: its high specific rotation and its reaction with alcohols in the presence of silver carbonate to yield β-glycosides are in accordance with this. Then α-glycoside formation cannot occur by the simple bimolecular process (route A), which involves a Walden inversion, although the small proportion of β-glycoside present in the products may be formed in this way. Nor is an anomerisation (A1) responsible for the formation of α-glycoside since it has been shown that no conversion of β- (VII) into α-glycoside (IV) occurs in chloroform at 50° in the presence of pyridine and pyridine hydrobromide.



A unimolecular reaction (route B, B1) would lead to β- or a mixture of α- and β-glycosides if neighbouring-group participation is not involved,[15] but it is questionable whether this

[10] For reviews see Lemieux, *Adv. Carbohydrate Chem.*, 1954, **9**, 1; Haynes and Newth, *ibid.*, 1955, **10**, 207.
[11] Isbell, *Ann. Rev. Biochem.*, 1940, **9**, 65; Winstein and Buckles, *J. Amer. Chem. Soc.*, 1942, **64**, 2780, 2787; Frush and Isbell, *J. Res. Nat. Bur. Stand.*, 1945, **34**, 111.
[12] Isbell and Frush, *J. Res. Nat. Bur. Stand.*, 1949, **43**, 161.
[13] Gorlin and Perlin, *Canad. J. Chem.*, 1961, **39**, 2427.
[14] Newth and Phillips, *J.*, 1953, 2904; 1957, 268; 1958, 130.
[15] Rhind-Tutt and Vernon, *J.*, 1960, 4637.

process would furnish very high yields of α-glycoside, and it is unlikely that the transitory formation of the carbonium ion (cation a) would be facilitated by the presence of a non-polar solvent.

Thus it becomes necessary to postulate an intermediate of β-configuration which will yield α-glycosides on reaction with alcohols by a process which involves a second inversion at C-1. The pyridinium compound (X) which is known to be formed during the reaction might appear as an appropriate intermediate. Nevertheless this cannot be so since compound (V) did not react with ethanol, even in the presence of pyridine and pyridine hydrobromide. It has also been shown in a similar way that neither the imine (XI) nor compound Q constitutes an intermediate in conversion of the bromide (VIII) into a glycoside.

A possible intermediate is the carbonium ion (cation b) or its pyridine-stabilised form, cation c (orthoacetate structures). The likelihood of stabilisation of carbonium intermediates by suitable nucleophiles has been recognised by other workers [12,16] and although neighbouring-group participation in reactions at position 1 is usually limited to 2-*trans*-substituents, participation of C-6 has been suggested to account for the synthesis of α-glycosides from Brigl's anhydride.[17] Of the three possible routes—B,B2,B3; B,B2 (cation b ⟶ cation c) C_1; and C,C_1—the last bimolecular process is at present considered the most likely since the ionic intermediate, cation c, which is a resonance-stabilised quaternary nitrogen derivative, is expected to be formed more readily in non-polar media than a carbonium ion, cation a or b. Moreover there is no evidence that orthoester derivatives are present in the final products, which would be expected if cation b were an intermediate. It will be seen that nucleophilic attack on cation c by an alcohol or the 2-substituent would produce an α-glycoside or the imine (XI). It is not clear why α-glycoside formation proceeding by route C should occur to a greater extent in non-polar media: possibly processes which lead to β-glycosides, such as routes A and B, are suppressed to a much greater extent than C in solvents of low dielectric constant.

In support of a mechanism involving the formation of a pyridine complex may be cited the observation that the bromide (VIII), unlike acetobromoglucose,[18] reacts with pyridine, as shown by a change in specific rotation when the two substances are mixed in chloroform. Addition of ethanol when the rotation becomes constant (about 30 min.) yields the α-glycoside (IV).

Silver Carbonate-catalysed Condensations.—The bromide (VIII) was also condensed with ethanol in the presence of silver carbonate and anhydrous calcium sulphate.[19] The results (Table 3) show the β-glycoside (VII) to be formed always in much higher yield than the α-glycoside (IV), although again rather more of the minor product (IV) was formed

TABLE 3.

Products from the bromide (VIII) and ethanol in the presence of silver carbonate (10 mol.) at room temperature (120 hours).

Solvent	Ethanol (mol.)	Yields (mg.) of (IV)	(VII)	(IX)	Ratio, IV/VII	Solvent	Ethanol (mol.)	Yields (mg.) of (IV)	(VII)	(IX)	Ratio, IV/VII
$CHCl_3$ ...	200	5·9	50·0	34·1	0·12	$MeNO_2$ ...	200	1·6	77·5	19·1	0·02
,, ...	3	4·7	37·5	51·9	0·13	,, ...	3	1·6	36·3	54·1	0·04

in chloroform and the highest yield of β-glycoside (IV) (83%) in the polar solvent (cf. Table 1). None of the other minor products of the pyridine-catalysed reactions was met in this series but there was always an appreciable quantity of the triacetate (IX). When only 3 mol. of alcohol were taken, more than 50% conversion into the hydrolysis

[16] Lemieux, *Adv. Carbohydrate Chem.*, 1954, **9**, 49.
[17] Lemieux, *Canad. J. Chem.*, 1953, **31**, 949.
[18] Goldschmid and Perlin, *Canad. J. Chem.*, 1961, **39**, 2025.
[19] Hammond and Withrow, *Ind. Eng. Chem.*, 1933, **25**, 653, 1112.

product (IX) resulted, in spite of the presence of a powerful desiccant. Undoubtedly the bromide (VIII) competes with the calcium sulphate for chemically produced water.

Mercuric Salt-catalysed Condensations.—In many cases the choice of mercuric salts as condensation agents in Koenigs–Knorr reactions has led to good yields of β-glucosides;[21,21] such reactions involved inversion of configuration at position 1 when the halogen atom was replaced by the alkoxy-group. However, it is equally known that the nature of the products formed in mercuric salt-catalysed reactions depends greatly on the conditions—in particular, selection of appropriate concentrations of alcohol and mercuric salt seems to facilitate formation of α-glycosides.[20,22]

Thus we studied also reactions of the bromide (VIII) with ethanol in the presence of mercuric acetate or bromide (see Table 4), the nature and amount of the catalyst, the solvent, and the concentration of the alcohol being varied. Column chromatography of the products was complicated by the presence in the α-glycoside band of an additional product which, by chromatographic comparison with an authentic specimen,[2] was shown to be 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-D-glucose (XII). To determine the quantities present in the composite band it was necessary to carry out a quantitative paper-chromatographic analysis. Preliminary experiments also indicated that when

TABLE 4.

Products from the bromide (VIII) (100 mg.) and ethanol in the presence of mercuric salts at 50°.

Salt nature	mol.	Solvent	Ethanol (mol.)	Duration (hr.)	(IV) (mg.)	(VII) (mg.)	(XII) (mg.)	(IX) (mg.)	Ratio, IV/VII
$Hg(OAc)_2$	1·0	$MeNO_2$	200	48	0·0	79·3	12·9	4·2	0·0
,,	,,	,,	3	,,	3·3	22·0	50·0	15·9	0·15
,,	,,	,,	1	,,	0·0	5·8	69·2	14·4	0·0
,,	,,	$CHCl_3$	200	,,	0·0	79·1	7·2	11·3	0·0
,,	,,	,,	3	,,	17·2	8·7	44·4	13·8	2·0
,,	,,	,,	1	,,	5·2	2·7	27·5	39·4	1·9
$Hg(OAc)_2$ *	0·5	$MeNO_2$	200	87	1·6	81·5	15·5	0·0	0·02
,,	,,	,,	3	,,	6·4	19·8	57·7	9·7	0·32
,,	,,	$CHCl_3$	200	,,	59·4	21·7	0·8	0·0	2·7
,,	,,	,,	3	,,	0·8	1·4	17·4	26·4	0·5
$HgBr_2$ *	1·0	$MeNO_2$	200	,,	23·8 †	33·2	4·0	32·8	(0·7)
,,	,,	,,	3	,,	67·5 ‡	1·8	7·6	9·4	—
,,	,,	$CHCl_2$	200	,,	69·4 †	5·9	0·4	0·0	(11·8)
,,	,,	,,	3	,,	9·4 †	9·1	15·9	0·0	(1·03)

* The products were acetylated before analysis. † Contained a trace of an unidentified compound. ‡ Contained a large amount of an unidentified compound.

mercuric acetate (0·5 mol.) and mercuric bromide were used there was extensive deacetylation; in these cases the products were acetylated before analysis. Nevertheless the triacetate (IX) could still be recognised in the final products: its presence may have been due to scission of the comparatively labile 1-acetoxy-group during chromatography on the "Magnesol" column.

Table 4 shows that the mercuric salt-catalysed reactions can furnish a high yield of either α- (IV) or β-glycoside (VII). The solvent effect is again important, polar solvents promoting the formation of the β-anomer, and only by using less than an equivalent amount of mercuric acetate is it possible with this catalyst to obtain a substantial yield of the α-glycoside.[23]

[20] Zemplén and Gerecs, *Ber.*, 1930, **63**, 2720,
[21] Lindberg, *Acta Chem. Scand.*, 1949, **3**, 151; Kuhn and Kirschenlohr, *Chem. Ber.*, 1953, **86**, 1331; Helferich, Doppstadt, and Gottschlich, *Naturwiss.*, 1953, **40**, 441; Helferich and Wedermeyer, *Annalen*, 1949, **563**, 139.
[22] Lindberg, *Arkiv Kemi, Mineral., Geol., B*, 1944, **18**, No. 9; Matsuda, *Chem. and Ind.*, 1958, 1627.
[23] Cf. Zemplén and Nagy, *Ber.*, 1930, **63**, 368.

Mercuric bromide-catalysed condensations afforded products which contained varying amounts of unidentified substances which moved on the chromatography column at the same rate as the α-glycoside (IV). Thus certain of the values in Table 4 are only approximate. It is concluded that, while the mercuric salt-catalysed reactions may lead to either α- or β-glycoside in good yield the pyridine- or silver carbonate-catalysed reactions will normally be preferred since, not only do they furnish the required glycoside in equally good yield, but also the pure product can be isolated more easily.

Application to α-Glycoside Synthesis.—Chromatographic separation of the products was rather time-consuming and therefore the possibility of obtaining good yields of α-glycosides by a more rapid procedure was explored. In optimum conditions the bromide (VIII) was treated in turn with methanol, ethanol, and benzyl alcohol in the presence of pyridine. The α-glycosides formed were isolated directly by recrystallisation of the products. Yields of ethyl, methyl, and benzyl α-glycoside were 78%, 62%, and 71%, respectively.

Experimental

Solvents.—Acetone was purified by being heated under reflux with small additions of potassium permanganate until the violet colour persisted. It was dried over anhydrous calcium sulphate, filtered, and fractionally distilled.

Acetonitrile was thrice distilled from phosphorus pentoxide and then fractionally distilled.

Chloroform was shaken with six changes of water (½ vol.), dried with calcium chloride, and distilled.

Dimethylformamide was dried with calcium chloride (24 hr.) and distilled.

Dioxan (1 l.), concentrated hydrochloric acid (14 ml.), and water (100 ml.) were refluxed for 7 hr., a slow stream of nitrogen being bubbled through the solution. It separated into two layers when solid potassium hydroxide was added and the dioxan layer was dried by being kept over solid potassium hydroxide, refluxed with sodium for 8 hr., and finally distilled in the presence of sodium.

Ethanol was dried by being refluxed with magnesium ethoxide and distilled.

Nitromethane was thrice distilled from phosphorus pentoxide and then fractionally distilled.

Pyridine was refluxed over sodium hydroxide for 2 hr. and then distilled. Thereafter it was refluxed over phosphorus pentoxide for 2 hr. and distilled.

Condensations were carried out under rigorously anhydrous conditions. Evaporation and distillation were carried out under diminished pressure unless otherwise stated.

Ethyl 2-Benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (I).—This was prepared from 2-benzyloxycarbonylamino-2-deoxy-D-glucose.[4] After prolonged drying over P_2O_5 at 70° it had m. p. 132—133°, $[\alpha]_D^{19}$ +100·6° (*c* 0·4 in EtOH). Foster, Horton, and Stacey [4] give m. p. 133°, $[\alpha]_D$ +100·6° (in EtOH).

Ethyl 2-Amino-2-deoxy-α-D-glucopyranoside Hydrochloride (II).—The aminoglucoside (I) (1·00 g.) in ethanol (30 ml.) containing hydrogen chloride (1 mol.) was reduced with hydrogen in the presence of 5% palladium–charcoal [3] (0·30 g.). When, after 3 hr., carbon dioxide was no longer evolved another portion of palladium–charcoal (0·30 g.) was added and reduction continued for 0·5 hr. The solution, when filtered and evaporated, gave an oil which slowly crystallised. Recrystallised several times from ethanol containing hydrogen chloride, *ethyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride* (0·18 g., 25%) had m. p. 197—198°, $[\alpha]_D^{19}$ +129·2° (*c* 0·4 in H_2O) (Found: N, 5·9. $C_8H_{18}ClNO_5$ requires N, 5·8%).

Ethyl 2-Deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranoside (III).—The hydrochloride (II) (0·18 g.) in water (6 ml.) containing sodium carbonate (0·19 g.) was added to a solution of 1-fluoro-2,4-dinitrobenzene (0·50 g.) in acetone (16 ml.). The mixture was shaken for 24 hr. at room temperature and the acetone removed under diminished pressure, leaving an orange paste, which was washed with water (35 ml.), dried, and recrystallised from ethanol–light petroleum. The yellow *product* (0·27 g., 97%) had m. p. 193—195°, $[\alpha]_D^{19}$ +25·9° (*c* 0·3 in acetone) (Found: C, 45·3; H, 5·0; N, 11·2. $C_{14}H_{19}N_3O_9$ requires C, 45·0; H, 5·1; N, 11·3%).

Ethyl 3,4,6-Tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranoside (IV).—The compound (III) (0·27 g.) was treated with pyridine (5 ml.) and acetic anhydride (2·5 ml.). After

24 hr. the solution was poured into ice-cold water, and after 30 min. the solid was collected, washed with water, and dried. Recrystallised from ethanol, the yellow *α-glycoside triacetate* (0·27 g., 75%) had m. p. 220—221°, $[\alpha]_D^{19}$ +14·8° (*c* 0·4 in $CHCl_3$) (Found: C, 48·3; H, 4·5; N, 8·6. $C_{20}H_{25}N_3O_{12}$ requires C, 48·1; H, 5·1; N, 8·4%).

Ethyl 3,4,6-*Tri*-O-*acetyl*-2-*amino*-2-*deoxy*-β-D-*glucopyranoside Hydrobromide* (VI).—This glycoside, obtained from the bromo-compound [6] (V), had m. p. 250° (decomp.), $[\alpha]_D^{19}$ +12·5° (*c* 0·7 in MeOH). Irvine and Hynd [6] give the same values.

Ethyl 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitroanilino*)-β-D-*glucopyranoside* (VII).—The glycoside (VI) (1·20 g.) and sodium carbonate (0·50 g.) in water (6 ml.) were treated with 1-fluoro-2,4-dinitrobenzene (0·55 g.) in acetone (20 ml.) as above. The *triacetate* of the β-glycoside (0·90 g., 62%) had m. p. 121—122° (from ethanol), $[\alpha]_D^{19}$ +3·5° (*c* 0·4 in $CHCl_3$) (Found: C, 48·1; H, 4·7; N, 7·8%).

3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitroanilino*)-α-D-*glucose* (IX).—3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide [2] (VIII), m. p. 164°, $[\alpha]_D^{18}$ +46·2° (*c* 0·3 in $CHCl_3$) (1·00 g.), was dissolved in acetone (40 ml.) containing water (6 ml.) and shaken with silver carbonate (5·0 g.) at room temperature for 36 hr. The solution was filtered and evaporated to dryness under diminished pressure and the crude product recrystallised from ethanol. The triacetate (0·78 g., 88%) had m. p. 154—156°, $[\alpha]_D^{18}$ −6·8° (*c* 0·5 in $CHCl_3$). Lloyd and Stacey [2] give m. p. 150—152·5°. An identical product was obtained (77%) when the bromo-compound (VIII) was treated with water, acetone, and pyridine for 7 days.

Identification, Separation, and Estimation of Dinitrophenyl Compounds.—(1) The solvent mixtures, (A) light petroleum (b. p. 60—80°)–acetone (5 : 1 by vol.), (B) light petroleum–acetone (10 : 1), and (C) light petroleum–acetic acid–acetone (8 : 1 : 1), were used to identify ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranoside (IV) (R_F 0·77, 0·57, 0·73), ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranoside (VII) (R_F 0·85, 0·75, 0·76), and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucose (IX) (R_F 0·37, 0·18, 0·18) (these R_F values refer, respectively, to the solvents A, B and C). The β-glycoside (VIII) gave discrete spots, but (IV) and (IX) gave elongated spots and showed streaking when the chromatogram was overloaded. The R_F values were not closely reproducible, and therefore when eluents from columns were examined, reference compounds were included in the chromatogram as controls.

Reverse-phase paper chromatography [24] and Wickberg's methods [25] were of little value on account of excessive streaking.

(2) When the α- (IV) and β-glycoside (VII) were deacetylated by treatment overnight with methanol saturated at 0° with ammonia, and the products were examined electrophoretically, each glycoside gave a single discrete spot. The deacetylated products were well separated by electrophoresis at 2000 v (potential gradient 40 v/cm.) in 0·1M-borate buffer (pH 10·0) or in 0·1M-ammonium molybdate buffer (pH 5·6). The triacetylglucosamine derivative (IX), on similar treatment, displayed a characteristic pattern of five discrete yellow spots none of which coincided with those derived from the ethyl glycoside (IV) or (VII) by deacetylation.

(3) Quantitative separation of the aminoglycosides (IV) and (VII) and the amino-sugar (IX) was carried out by chromatography on columns of "Magnesol" and "Celite." "Magnesol" and "Celite" were mixed together in the weight ratio of 5 : 1. The mixture (60 g.) was activated by being stirred with acetone (330 ml.), filtered at the pump, and dried by suction for 1 hr., and then in air at 35—40° for 20 hr. A slurry of the powder (25 g.) in benzene (100 ml.) was poured into the column, which was washed with 2 column-lengths of benzene. A mixture to be analysed was dissolved in a minimum quantity of benzene and introduced at the top of the column. Elution was with benzene–acetone (50 : 1), and fractions (10 ml.) were collected in an "Aimer" automatic fraction-collecter. Fractions corresponding to each separate yellow band were combined and made up to a known volume, and an aliquot part was withdrawn and evaporated to dryness. The residue was dissolved in a known volume of ethanol and the optical density of the solution at 340 mμ [26] was measured in a Unicam S.P. 500 spectrophotometer. By reference to the calibration curves (see Table 5), the amount of material in each band was estimated. The identity of each band was established by its

[24] Micheel and Albess, *Mikrochim. Acta*, 1954, 489.
[25] Wickberg, *Acta Chem. Scand.*, 1958, **12**, 615.
[26] Annison, James, and Morgan, *Biochem. J.*, 1951, **48**, 477; Foster, Martlew, and Stacey, *Chem. and Ind.*, 1953, 899.

characteristic position on the column (elution occurred in the order: IV, VII, IX) and by methods (1) and (2) above.

In a test run the glycosides (IV) (0·100 g.) and (VII) (0·100 g.) were mixed and separated on a column (45 × 2·5 cm.). Spectrophotometric determinations indicated recoveries of 96·3% and 100·6%, respectively.

TABLE 5.

Concn. (mg./100 ml.) in EtOH	Optical density at 340 mμ.				
	IV	VII	IX	XI	XII
0·2	0·082	0·077	0·062	0·054	0·076
0·5	0·184	0·173	0·156	0·147	0·179
1·0	0·361	0·340	0·305	0·309	0·338
1·4	0·508	0·475	0·422	0·421	0·478
2·0	0·715	0·675	0·604	0·599	0·672

Condensation of 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitroanilino*)-α-D-*glucopyranosyl Bromide* (VIII) *with Ethanol in the Presence of Pyridine.*—The bromide (VIII) (0·100 g.) in a solvent (Tables 1 and 2) containing ethanol (3—200 mol.) and pyridine (0·030 ml., 2 mol.) was kept at 50° until reaction was complete. The solvent was evaporated and the residue, dissolved in benzene [a small quantity of the pyridinium compound (X) remained undissolved], was filtered and transferred quantitatively to a " Magnesol "–" Celite " column (45 × 2·5 cm.). Elution, identification, and estimation of the products were carried out as described above. In certain cases (see Discussion) the first band to be eluted was not the α-glycoside (IV) but a weak band which on elution and evaporation yielded 3,4,6-*tri*-O-*acetyl*-1,2-*dideoxy*-1,2-(2,4-*dinitrophenyl*)-*epi*-*imino*-α-D-*glucopyranose* (XI). Recrystallised from ethanol this formed the *diethanolate*, m. p. 175—177°, $[\alpha]_D^{18}$ +1·4° (*c* 0·4 in $CHCl_3$) [Found: C, 49·2; H, 5·6; N, 7·5; OAc, 33·2%; *M*(Rast), 523. $C_{22}H_{31}N_3O_{13}$ requires C, 48·4; H, 5·7; N, 7·7; OAc, 32·5%; *M*, 545].

In the absence of the imine (XI), the first band to be eluted from the column contained the α-glycoside (IV). Following this band and preceding the β-glycoside (VII), a faint band was eluted in some cases which on evaporation yielded yellow crystals (Q), m. p. 193° (decomp.), $[\alpha]_D^{18}$ +5·0° (*c* 0·28 in $CHCl_3$), which migrated towards the anode on the electrophoretogram and decomposed in the presence of light to form a white substance (R), insoluble in benzene, which was not investigated.

Preliminary experiments were carried out to determine the time required for the completion of the condensation reaction. The bromide (VIII), dissolved in the specified solvent (4 ml.), was treated with ethanol (3 mol.) in the presence of pyridine (2 mol.) at different temperatures. The reactions, followed polarimetrically, were complete after 13 hr. (in dioxan at 75°), 24 hr. (in acetone at 50°), or 215 hr. (in acetone at 18°).

When the bromide (0·20 g.) in chloroform (8·00 ml.) was treated with pyridine (0·061 ml.) at 50° the optical rotation (0·5 dm. tube) of the solution changed from +0·62° (initial) to +0·45° (30 min.) (constant).

1-[3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitroanilino*)-β-D-*glucopyranosyl*]*pyridinium Bromide* (X).—This was formed in low yield (2—10%) in the pyridine-catalysed reactions described above and was also prepared as follows. The bromide (VIII) (1·00 g.) was heated in pyridine (40 ml.) and ethanol (21·9 ml.) at 50° for 24 hr. and then evaporated. The residue was stirred with a large volume of benzene. The insoluble component on recrystallisation from ethanol–benzene or n-propyl alcohol–dioxan furnished yellow needles of the *pyridinium compound* (0·95 g., 83%), m. p. 162·5° (decomp.), $[\alpha]_D^{18}$ −145·6° (*c* 0·2 in H_2O) (Found: C, 44·5; H, 4·3; N, 9·2; Br, 13·8. $C_{23}H_{25}BrN_4O_{11}$ requires C, 45·0; H, 4·1; N, 9·1; Br, 13·0%).

Condensation of the Bromide (VIII) *with Ethanol in the Presence of Silver Carbonate.*—The bromo-compound (0·100 g.) was dissolved in the specified solvent (Table 3) containing ethanol (3 or 200 mol. in total volume of 6·00 ml.), freshly prepared, dry silver carbonate (0·517 g., 10 mol.), and activated anhydrous calcium sulphate (0·20 g.). The mixture was shaken in the dark at room temperature (calcium chloride guard-tube) until the reaction was complete as indicated by the absence of bromine from the supernatant liquid. The solution was then filtered through charcoal, which was washed until the filtrate was no longer coloured. The

filtrate and washings were evaporated to dryness, and the residue was dissolved in benzene and analysed as described above.

Condensation of the Bromide (VIII) *with Ethanol in the Presence of Mercuric Salts.*—The bromo-compound (0·100 g.) was dissolved in the solvent containing ethanol and the mercuric salt (total volume 6·00 ml.) in a stoppered flask and kept at 50° for the times recorded in Table 4. The solution was evaporated, and the residue was dissolved in chloroform (15 ml.) and washed with water (30 ml.). The chloroform solution was evaporated, and the residue dissolved in benzene and analysed on a column as described above. The band containing the β-glycoside (VII) usually contained also the tetra-acetate (XII). These products were separated by paper chromatography in light petroleum–acetone (7 : 1). After development the chromatogram was dried and spots corresponding to the products (VII) and (XII) were cut from the paper and shaken with ethanol (4·00 ml.) for 1 hr. Quantitative transfer occurred from paper to ethanol and measurement of the optical densities at 340 mμ gave the relative proportions (Table 4). The total amount of material in the original band was determined spectrophotometrically.

Frequently the mixture resulting from the condensation was acetylated. In such a case, the mixture was evaporated and the residue treated with pyridine (12 ml.) and acetic anhydride (4 ml.) at room temperature for 24 hr. The acetylated product was isolated in the usual manner and analysed chromatographically.

Synthesis of α-Glycosides from the Bromide (VIII).—The bromide (0·80) was dissolved in chloroform (31 ml.) containing ethanol (17·5 ml.) and pyridine (0·24 g.) and heated at 50° for 26 hr. Recrystallisation of the residue obtained when the solution was evaporated gave ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glycopyranoside (IV) (0·58 g., 78%), m. p. and mixed m. p. 220—221°, $[\alpha]_D^{18}$ +15·6° (*c* 0·4 in $CHCl_3$). In a similar manner were prepared the corresponding α-benzyl glycoside (71%), m. p. 210—211°, $[\alpha]_D^{18}$ +6·4° (*c* 0·6 in $CHCl_3$) {Lloyd and Stacey [2] give m. p. 198—200°, $[\alpha]_D^{19}$ +9·0° (*c* 0·3 in $CHCl_3$)}, and α-methyl glycoside (62%), m. p. 206—207·5°, $[\alpha]_D^{18}$ +1·8° (*c* 0·4 in $CHCl_3$) {Wang Yu and Tai Hsing [27] give m. p. 180—182°, $[\alpha]_D$ +11° (in $CHCl_3$); Lloyd and Stacey [2] give m. p. 206°, $[\alpha]_D^{19}$ +21·3° (*c* 0·5 in $CHCl_3$)}.

Methyl 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-(2,4-*dinitroanilino*)-β-D-*glucopyranoside.*—This compound, prepared by Lloyd and Stacey's method,[2] has now been obtained crystalline in 74% yield. After recrystallisation from aqueous methanol it had m. p. 68—77°, $[\alpha]_D^{19}$ +14·0° (*c* 0·3 in $CHCl_3$). After further purification on a " Magnesol "–" Celite " column and recrystallisation it had m. p. 70—75°, $[\alpha]_D^{18}$ +14·4° (*c* 0·4 in $CHCl_3$).

This β-glycoside was also prepared from the bromide (VIII) (with silver carbonate as catalyst). The product, obtained in 18% yield, had m. p. 79—81°, $[\alpha]_D^{18}$ +16·4° (*c* 0·5 in $CHCl_3$). Wang Yu and Tai Hsing [27] give m. p. 78—80°, $[\alpha]_D$ +34° (in $CHCl_3$).

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[27] Wang Yu and Tai Hsing, *Acta Chim. Sinica*, 1958, **24**, 368.