551. *Chemical Studies in the Biosynthesis of Purine Nucleotides. Part I. The Preparation of* N-*Glycylglycosylamines.*

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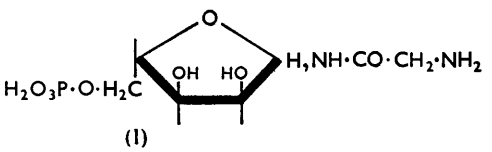
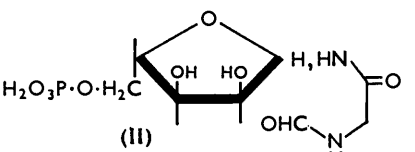
Derivatives of *N*-glycylglucosylamine have been prepared in preliminary experiments directed towards the synthesis of " glycineamide ribotide " (I) and its formyl derivative (II), two early nucleotide precursors. Tetra-*O*-acetyl-β-D-glucopyranosylamine (III) with chloroacetyl chloride gave the *N*-chloroacetyl derivative (V). Replacement of the chloro- by an amino-group and simultaneous deacetylation, by means of ammonia, gave *N*-glycyl-β-D-glucopyranosylamine (VI) which was readily converted into its *N*-formyl derivative. An alternative synthesis of the glycyl compounds involves reaction between the amino-sugar and benzyloxycarbonylglycyl chloride or benzyloxycarbonylglycyl ethyl carbonate, followed by removal of protecting groups.

It has been shown by J. M. Buchanan and his collaborators that the uric acid excreted by pigeons is built up from simple substances supplied in the diet. By feeding isotopically labelled compounds and studying the distribution of isotopes in the uric acid formed, it was found that the carbon and nitrogen atoms of the carboxyl, methylene, and amino-groups of glycine were incorporated as a unit into positions 4, 5, and 7 respectively in uric acid. The carbon at positions 2 and 8 originates from formate, and that at position 6 from carbon dioxide.[1-4] The origin of the nitrogen at positions 1, 3, and 9 was not determined conclusively but recent evidence suggests that the atoms at positions 3 and 9 come from the amide-nitrogen of glutamine and that at position 1 from the α-amino-group of aspartic or glutamic acid.[5]

In pigeon-liver extracts hypoxanthine (6-hydroxypurine) is formed rather than uric acid and it is generally considered that this is the normal precursor of uric acid produced by the living bird. It was shown by Greenberg that the hypoxanthine arose through enzymic hydrolysis of inosinic acid and that the ribose-5 phosphate residue in this compound was attached at quite an early stage in its biosynthesis.[6-8] Although all the stages in the



(I)



(II)

formation of inosinic acid from ribose-5 phosphate, glycine, etc., have not yet been clarified, several of the intermediates have now been isolated or detected chromatographically.[5,7,9]

Recent evidence suggests that *N*-glycyl-D-ribofuranosylamine-5 phosphate (" glycineamide ribotide ") (I) is formed from glycine, glutamine, and 5-phosphorylribose-1 pyrophosphate (PRPP) in the presence of enzymes in pigeon liver.[10,11] The stages involved in this synthesis are not yet settled, but it appears that glutamine contributes its amide-nitrogen to form the amide-nitrogen of glycineamide ribotide, and that adenosine

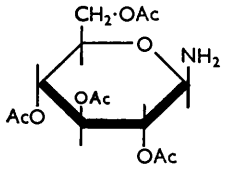
[1] Sonne, Buchanan, and Delluva, *J. Biol. Chem.*, 1946, **166**, 395.
[2] *Idem, ibid.*, 1948, **173**, 69.
[3] *Idem, ibid.*, p. 81.
[4] Buchanan and Sonne, *ibid.*, 1946, **166**, 781.
[5] Buchanan and Wilson, *Fed. Proc.*, 1953, **12**, 648; and personal communication.
[6] Greenberg, *ibid.*, 1950, **9**, 179.
[7] *Idem, ibid.*, 1953, **12**, 651.
[8] *Idem, J. Biol. Chem.*, 1951, **190**, 611.
[9] *Idem, Fed. Proc.*, 1954, **13**, 745.
[10] Goldthwait, Peabody, and Greenberg, *J. Amer. Chem. Soc.*, 1954, **76**, 5258.
[11] Hartman, Levenberg, and Buchanan, *ibid.*, 1955, **77**, 501.

triphosphate is required in the synthesis. Subsequent stages include the conversion of glycineamide ribotide into its *N*-formyl derivative (II) and cyclisation of this to glyoxaline derivatives bearing a ribofuranose-5 phosphate residue. Eventually, a ribofuranose-5 phosphate derivative of 5-aminoglyoxaline-4-carboxyamide is formed and this may be formylated enzymically and cyclised to inosinic acid.[12]

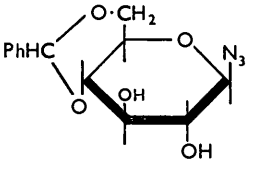
Although other enzymic routes to purine nucleotides exist, *e.g.*, from preformed purines or from 5-aminoglyoxaline-4-carboxyamide and ribose phosphates, there is no evidence to suggest that either free purines or the glyoxaline is built up as such in living systems, and it is generally believed that the above route through glycine amide derivatives of ribose-5 phosphate represents the main pathway of purine-nucleotide biosynthesis. It is essential then, for a fuller understanding of this route, that the structure of the intermediates be rigidly established. It is our hope in this series of papers to be able to confirm by synthesis some of the tentative structures assigned to the intermediates which have been isolated from cell extracts, and to prepare possible intermediate substances which have not yet been detected in the biological systems.

The object of the work described now was to develop general syntheses of *N*-glycylglycosylamines and their derivatives in order to clarify certain structural features in the natural *N*-glycylribofuranosylamines. It is not known, for example, whether the natural compounds are α- or β-glycosides, and there has been some uncertainty regarding the location of the formyl group in the derivative (II). *N*-Acyl derivatives of glycosylamines have received rather little attention and no satisfactory general methods for their preparation are recorded. We have examined two possible routes. The first would involve reaction between a derivative of an amide and a suitably substituted sugar, whereas the second would depend on *N*-acylation of a 1-amino-1-deoxy-sugar (glycosylamine). Attempts to prepare *N*-acylglucosylamines by reaction between metal salts of amides and acetylglucosyl bromide were rather unsatisfactory and our attention was directed to syntheses from glucosylamine derivatives.

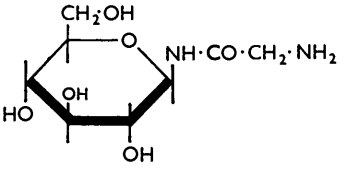
Tetra-*O*-acetyl-β-D-glucopyranosylamine (III) was considered to be a suitable model. This may be prepared by hydrogenolysis of either tetra-*O*-acetyl-*N*-benzyl-β-D-glucopyranosylamine [13] or tetra-*O*-acetyl-β-D-glucopyranosyl azide.[14] We found the second method more satisfactory. With methanolic ammonia the acetylated azide yields β-D-glucopyranosyl azide.[15] Although in our hands this did not crystallise,[16] it appeared to be homogeneous on paper chromatography and readily gave a crystalline 4 : 6-*O*-benzylidene derivative (IV), from which acid hydrolysis regenerated β-D-glycosyl azide, identified chromatographically. It is interesting that the β-azide is rather stable towards hydrolysis and ammonolysis.



(III)



(IV)



(VI)

RNH·CO·CH₂Cl (V)

RNH·CO·CH₂NH·CO·OCH₂Ph (VII)

RNH·CO·NH·CH₂·NH·CO·OCH₂Ph (VIII)

(R = tetra-*O*-acetyl-β-D-glucopyranosyl)

Several methods for the preparation of *N*-glycyl-β-D-glucopyranosylamine (VI) from the amine (III) were developed. With chloroacetyl chloride a good yield of tetra-*O*-acetyl-*N*-chloroacetyl-β-D-glucopyranosylamine (V) was obtained. With ammonia in

[12] Buchanan and Schulman, *J. Biol. Chem.*, 1953, **202**, 241.
[13] Helferich, *Chem. Ber.*, 1953, **86**, 603.
[14] Bertho and Maier, *Annalen*, 1932, **498**, 50.
[15] Bertho, *Ber.*, 1930, **63**, 836.
[16] Micheel, Klemer, and Baum, *Chem. Ber.*, 1955, **88**, 475.

methanol this gave the desired glycyl compound which was characterised by its behaviour on paper chromatography and as its crystalline oxalate and picrate. A more general synthesis of *N*-glycylglycosylamines consists in reaction between the appropriate acylated glycosylamine and benzyloxycarbonylglycyl chloride or benzyloxycarbonylglycyl ethyl carbonate. In both cases reasonable yields of the benzyloxycarbonylglycyl compound (VII) were obtained from tetra-*O*-acetyl-β-D-glucopyranosylamine, the acid chloride route being preferred.[17] Acetyl groups were removed by treatment with sodium methoxide in methanol, and the benzyloxycarbonyl residue by catalytic hydrogenolysis, to give *N*-glycyl-β-D-glucopyranosylamine (VI).

The tetra-*O*-acetylglucosyl azide has the β-configuration, both forms having been prepared by Bertho *et al.*[15,18] Also, neither the *N*-glycylglucosylamine nor the crystalline *N*-acylated intermediates in its synthesis showed any tendency to mutarotate; consequently it is reasonable to assume that they are all β-pyranosides. The stability of the *N*-glycylglucosylamine towards mutarotation is supported by the observation that it consumed exactly 2 mols. of periodate at neutral pH. In this respect it differs from glucosylamines of the phenyl or pyrimidinyl series and resembles *N*-acetylglucopyranosylamine.[19]

Benzyloxycarbonylglycyl azide reacted readily with tetra-*O*-acetyl-β-D-glucopyranosylamine (III), but paper chromatography of the deacetylated product showed that a mixture had been formed. Although some of the desired benzyloxycarbonylglycyl derivative (VII) was present, the main product was a crystalline compound giving analytical figures more or less consistent with those for the urea (VIII). This substance was not obtained analytically pure but its formation is analogous to the production of ureas from benzyloxycarbonylglycyl azide and derivatives of glucosamine.[20]

N-(Formylglycyl)-β-D-glucopyranosylamine, analogous to the naturally occurring formyl compound (II), was readily prepared in good yield by treatment of the glycyl compound (VI) with formic acid in acetic anhydride and then with methanolic ammonia to remove *O*-formyl residues.

Both *N*-glycylglucopyranosylamine and its formyl derivative resembled the purine nucleotide precursors (I) and (II) respectively in their behaviour towards the ninhydrin spray reagent on paper. Whereas the synthetic *N*-glycyl compound (VI) and the natural precursor (I) give positive reactions, the formyl derivatives do not. Syntheses along similar lines in the D-ribofuranose series are in progress.

In the Experimental section we describe a greatly improved Schiff reagent for use in the detection of α-glycols after oxidation on paper by sodium metaperiodate.

Experimental

Unless otherwise stated, solvents were removed under reduced pressure at $<40°$ (bath).

Paper Chromatography.—Two solvent systems were used, *viz.*, the water-poor phase from butan-1-ol–acetic acid–water (4 : 1 : 5 v/v) and propan-1-ol–aqueous ammonia (*d* 0·880)–water (6 : 3 : 1 v/v).[21] Irrigation was by the ascending technique on Whatman No. 4 paper. Three spray reagents were used: ninhydrin, chlorine–iodide–starch,[22] and periodate–Schiff's reagent.[23] The last technique has been modified as follows: the dried paper is sprayed with 1% aqueous sodium metaperiodate, left for 5—10 min., treated with sulphur dioxide, and then sprayed with Schiff's reagent [a 1% suspension of pararosaniline hydrochloride (Hopkin and Williams, Ltd.) treated with sulphur dioxide until a pale straw-coloured solution results]. The colours are allowed to develop at room temperature. When this method is used it is not necessary to carry out the oxidation in a nitrogen atmosphere, even for compounds which yield

[17] Cf. Baker, Joseph, and Williams, *J. Amer. Chem. Soc.*, 1955, **77**, 1.
[18] Bertho and Aures, *Annalen*, 1955, **592**, 54.
[19] Niemann and Hays, *J. Amer. Chem. Soc.*, 1940, **62**, 2960
[20] Popenhoe, Doherty, and Link, *ibid.*, 1953, **75**, 3469.
[21] Hanes and Isherwood, *Nature*, 1949, **164**, 1107.
[22] Rydon and Smith, *ibid.*, 1952, **169**, 922.
[23] Buchanan, Dekker, and Long, *J.*, 1950, 3162.

only formaldehyde on oxidation (cf. ref. 23). The sensitivity compares very favourably with that of the periodate–benzidine method.[24]

2 : 3 : 4 : 6-*Tetra*-O-*acetyl*-N-*chloroacetyl*-β-D-*glucopyranosylamine*.—2 : 3 : 4 : 6-Tetra-*O*-acetyl-β-D-glucopyranosylamine (m. p. 125—127°) (3·0 g., 1 mol.) was dissolved in dry chloroform (20 c.c.) and cooled to 0°. Chloroacetyl chloride (0·32 c.c.; 0·5 mol.) was added slowly and the mixture left at 0° overnight. Amine hydrochloride was removed by filtration and washed with chloroform. The combined chloroform solution was washed with water, dried by distillation at atmosphere pressure to 10 c.c., and diluted with light petroleum (b. p 60—80°) to 40 c.c. On cooling, the crystalline product separated (m. p. 166—167·5°; 1·45 g., 79% based on recovery of base hydrochloride). Recrystallised from 95% ethanol (15 c.c.) the N-*chloroacetyl compound* had m. p. 166·5—167·5°, $[\alpha]_D^{19}$ +10·8° (*c* 1·8 in $CHCl_3$) (Found : C, 45·2; H, 5·3; N, 3·3; Cl, 8·6. $C_{16}H_{22}O_{10}NCl$ requires C, 45·4; H, 5·2; N, 3·3; Cl, 8·4%).

2 : 3 : 4 : 6-*Tetra*-O-*acetyl*-N-(*benzyloxycarbonylglycyl*)-β-D-*glucopyranosylamine*.—(a) *Acid chloride method.* 2 : 3 : 4 : 6-Tetra-*O*-acetyl-β-D-glucosylamine (m. p. 125—127°) (11·2 g., 1 mol.) was dissolved in dry chloroform (60 c.c.) and cooled to 0°. Benzyloxycarbonylglycyl chloride (12·5 g., 1·75 mol.) was added, the mixture kept at 0° for 15 min., pyridine (14 c.c., 5 mols.) added, and the mixture left at 0° for 1 hr. The chloroform solution was washed with 2N-hydrochloric acid, water, saturated sodium hydrogen carbonate solution, and water, then dried (Na_2SO_4), filtered and evaporated to a yellowish oil. Crystallisation from ethanol gave 6·5 g., m. p. 123—125°, the mother-liquors on concentration yielding a further 3·2 g., m. p. 118—120°. The crystals contained ethanol of crystallisation (total yield 52%). Recrystallised from ethanol the *benzyloxycarbonyl compound* had m. p. 127—127·5°, $[\alpha]_D^{19}$ +8·5° (*c* 2·0 in EtOH) (Found : C, 53·2; H, 6·2; N, 5·1. $C_{24}H_{30}O_{12}N_2,C_2H_5{\cdot}OH$ requires C, 53·4; H, 6·2; N, 4·8%).

(b) *Mixed carbonic anhydride method.* Ethyl chloroformate (2·4 g., 1 mol.) was added dropwise to a solution of benzyloxycarbonylglycine (4·62 g., 1 mol.) and triethylamine (2·25 g., 1 mol.) in dry chloroform (30 c.c.) at −20°. The solution was allowed to reach −5° and kept at that temperature for 10 min., while triethylamine hydrochloride separated. A solution of 2 : 3 : 4 : 6-tetra-*O*-acetyl-β-D-glucopyranosylamine (7·6 g., 1 mol.) in dry chloroform (60 c.c.) at −5° was added to the above mixture and kept at this temperature for 30 min. and then at room temperature for 2 hr. The triethylamine hydrochloride redissolved during 10 min. and carbon dioxide was evolved for the first 25 min. The chloroform solution was washed, dried, and evaporated as in (a) above, yielding from ethanol (20 c.c.) the product (4·26 g.; m. p. 123—125°). From the mother-liquors a further 0·57 g. was isolated (total yield 41%). Recrystallisation from ethanol raised the m. p. to 127—127·5°, undepressed when mixed with the product from (a).

N-(*Benzyloxycarbonylglycyl*)-β-D-*glucopyranosylamine*.—2 : 3 : 4 : 6-Tetra-*O*-acetyl-*N*-(benzyloxycarbonylglycyl)-β-D-glucopyranosylamine (4·0 g., 1 mol.) in dry methanol (50 c.c.) was mixed with a solution of sodium (0·017 g., 0·1 mol) in methanol (5 c.c.) and kept for 24 hr. at room temperature. Amberlite IRC-50 resin (H^+ form; washed with methanol, *ca.* 5 g.) was added to neutralise the alkali. The resin was filtered off and washed with methanol, and the combined filtrates were evaporated to 25 c.c. The crystalline product separated, on seeding, as plates, m. p. 178—179·5° (1·75 g.), and the mother-liquors yielded a further 0·45 g., m. p. 177—178° (total yield, 80%). Recrystallisation from dry methanol gave plates, m. p. 178—180°, $[\alpha]_D^{18}$ −9·0° (*c* 1·9 in EtOH) (Found : C, 51·5; H, 6·2; N, 7·8. $C_{16}H_{22}O_8N_2$ requires C, 51·9; H, 6·0; N, 7·6%).

N-*Glycyl*-β-D-*glucopyranosylamine Hydrogen Oxalate*.—(a) *From the chloroacetyl compound.* 2 : 3 : 4 : 6-Tetra-*O*-acetyl-*N*-chloroacetyl-β-D-glucopyranosylamine (2·1 g.) was dissolved in methanol (30 c.c.; saturated with ammonia at 0°) and kept at room temperature for 75 hr. The methanol and ammonia were removed by distillation and the resulting syrup was extracted thrice with hot benzene to remove acetamide. The syrup was dissolved in water (5 c.c.) and passed slowly (0·5 c.c. per min.) through a column of Amberlite IR-4B resin (OH^- form; 1 cm. × 5 cm.), followed by water until the effluent had a negative Molisch reaction. The combined effluent was concentrated to a syrup which was dissolved in water (5 c.c.) and passed through a column of Amberlite IRC-50 (H^+ form; 1 cm. × 5 cm.). Washing with water was continued until the Molisch reaction was negative, and the combined effluent was recycled through the IR-4B and the IRC-50 column until the effluent was neutral. A solution of oxalic acid (1·5 g. of dihydrate) in water (30—40 c.c.) was then passed through the Amberlite IRC-50 column until the effluent gave no Molisch test. The effluent was concentrated to 5 c.c. and ethanol

[24] Cifonelli and Smith, *Analyt. Chem.*, 1954, **26**, 1132.

(20—30 c.c.) added. Crystals were formed. They were dissolved by heat and then the solution was treated with charcoal and filtered. On cooling, the crystalline *oxalate* (750 mg.) separated and was filtered off, washed with ethanol, and dried. It had m. p. 195—197° (decomp.) (Found : N, 8·1. $C_{10}H_{18}O_{10}N_2,H_2O$ requires N, 8·1%). The oxalate consumed 2·02 mol. of sodium metaperiodate in 42 hr. (no further uptake after 70 hr.).

(b) *From the benzyloxycarbonyl compound.* *N*-Benzyloxycarbonylglycyl-β-D-glucopyranosylamine (0·76 g., 1 mol.) was hydrogenolysed in water (10 c.c.) and ethanol (45 c.c.) with palladium (10%) as catalyst. The carbon dioxide evolved was absorbed by a concentrated sodium hydroxide solution in a suitably protected side-arm. Hydrogen uptake was complete (95%) in 4 hr. The solution was filtered from the catalyst, which was washed with aqueous alcohol. A solution of oxalic acid in ethanol was added and the precipitated product collected. After recrystallisation from ethanol–water it (0·31 g., 45%) had m. p. 196—196·5°, $[\alpha]_D^{18}$ −11·1° (*c* 2·0 in H_2O). The m. p. was undepressed in admixture with a sample from (a) above. The behaviour on paper chromatography was also identical (Found : C, 34·3; H, 6·1. $C_{10}H_{18}O_{10}N_2,H_2O$ requires C, 34·9; H, 5·8%). In later preparations the yield was increased by using an ion-exchange column as in (a) above.

N-*Glycyl*-β-D-*glucosylamine Picrate.*—The procedure was the same as for the oxalate [route (*a*)], but the final elution from the Amberlite IRC-50 column was effected with picric acid. The *picrate*, crystallised from ethanol, had m. p. 189—195° (decomp.) (700 mg. from 3 g. of tetra-*O*-acetyl-β-D-glucopyranosylamine), but 205—207° (decomp.) after recrystallisation from aqueous ethanol (Found : C, 35·2; H, 4·5; N, 14·8. $C_{14}H_{19}O_{13}N_5,H_2O$ requires C, 35·5; H, 4·5; N, 14·8%).

Paper chromatography of oxalate and picrate. These gave one spot with the ninhydrin, periodate–Schiff, and chlorine–iodide–starch reagents in both solvent systems. For R_F values, see Table.

R_F values.

Compound	Ninhydrin	IO_4^-/Schiff	Cl_2–KI	BuOH–AcOH	PrOH–NH_3
N-Glycyl-β-D-glucosylamine	+ (Yellow)	+	+	0·13	0·51
β-D-Glucosyl azide	—	+	—	0·52	0·75
N-(Formylglycyl)-β-D-glucosylamine	—	+	+	0·20	0·56
N-(Benzyloxycarbonylglycyl)-β-D-glucosylamine	—	+	+	0·63	0·90
Glycine	+ (Purple)	— *	+	0·18	0·51
Glucose	—	+	—	0·21	0·59

* A red spot was sometimes formed. This effect has been noted with certain basic compounds,[23] and is quite distinct from the normal purple spot.

Acid hydrolysis. Small portions of the oxalate were treated separately with 0·12N-hydrochloric acid and 1·2N-hydrochloric acid. Paper chromatography showed that 0·12N-acid had caused no hydrolysis in 4 hr. With 1·2N-acid, however, hydrolysis to glycine and glucose had occurred (*ca.* 50% after 1 hr.; *ca.* 80—90% after 4 hr.).

N-(*Formylglycyl*)-β-D-*glucopyranosylamine.*—*N*-Glycyl-β-D-glucopyranosylamine hydrogen oxalate monohydrate (200 mg.) was dissolved in 98% formic acid (5 c.c.), and a mixture of 98% formic acid (15 c.c.) and acetic anhydride (5 c.c.) was added at 30—40°. The whole was kept at 30—40° for 1 hr. and then left overnight at 0°. Evaporation left a syrup which was evaporated with several lots of ethanol. The residue was dissolved in methanol (20 c.c.; saturated with ammonia at 0°) and left overnight at 0°. The solution was evaporated to a syrup which was dissolved in water and passed successively through columns of Amberlite IR-4B (OH^- form; 1 cm. × 5 cm.) and Amberlite IRC-50 (H^+; 1 cm. × 5 cm.) which were washed as before until the final effluent was neutral. This effluent was concentrated to a syrup, and ethanol added to give the crystalline *formyl compound*, m. p. 214—215° (120 mg., 69%). Recrystallisation from aqueous methanol raised the m. p. to 215·5—216·5°, $[\alpha]_D^{18}$ −13·7° (*c* 2·3 in H_2O) (Found : C, 41·0; H, 6·3; N, 10·6. $C_9H_{16}O_7N_2$ requires C, 41·1; H, 6·1; N, 10·7%).

Acid hydrolysis. A few crystals of formyl compound were dissolved in a small quantity of 0·12N-hydrochloric acid and left at room temperature for 1 hr. Paper chromatography showed complete conversion into *N*-glycyl-β-D-glucopyranosylamine. For R_F values, see Table.

4 : 6-O-*Benzylidene*-β-D-*glucopyranosyl Azide.*—2 : 3 : 4 : 6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide (1·5 g.) was treated with saturated methanolic ammonia (30 c.c.) at room temperature for 2 days. The syrup obtained by evaporation of the solvent was passed through columns of Amberlite IR-4B (OH^- form; 1 cm. × 5 cm.) and Amberlite IRC-50 (H^+ form;

1 cm. × 5 cm.) with aqueous washing. Removal of solvent left a syrup. Part of the syrup was shaken with freshly distilled benzaldehyde (5 c.c.) and powdered anhydrous zinc chloride (800 mg.) for 20 hr. The product was shaken with excess of saturated potassium carbonate solution and filtered, and the zinc carbonate washed with water, acetone, and ethanol. The filtrate was concentrated to a semi-solid mass, water and ethanol were added, and the evaporation was repeated. The residue was extracted with hot ethanol. Crystals of potassium benzoate separated on cooling. These were removed by filtration, the filtrate was evaporated to dryness, and the residue on treatment with water gave the *benzylidene compound* as needles, m. p. 150—151° (decomp.). Recrystallisation from ethyl acetate–light petroleum (b. p. 60—80°) raised the m. p. to 153—154°, $[\alpha]_D^{18}$ −65·7° (*c* 0·5 in EtOH) (Found: C, 53·0; H, 5·2; N, 13·9. $C_{13}H_{15}O_5N_3$ requires C, 53·2; H, 5·2; N, 14·3%).

Acid hydrolysis. A few crystals were treated with 0·1N-hydrochloric acid in 50% aqueous ethanol for 24 hr. Paper chromatography showed their conversion into β-D-glucopyranosyl azide together with a trace of glucose.

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