55. *Synthesis of* 2-O-α-D-*Glucosaminylglycerol and* 2-O-β-D-*Glucosaminylglycerol.*

By F. E. Hardy.

Both 2-*O*-α- and 2-*O*-β-D-glucosaminylglycerol have been synthesised by a Koenigs–Knorr reaction from 3,4,6-tri-*O*-acetyl-*N*-4-methoxybenzylidene-α-D-glucosaminyl bromide and 1,3-di-*O*-benzylglycerol; only the β-anomer was obtained when 3,4,6-tri-*O*-acetyl-*N*-diphenoxyphosphinyl-α-D-glucosaminyl bromide was used.

In a previous Paper [1] it was reported that treatment of 3,4,6-tri-*O*-acetyl-*N*-4-methoxybenzylidene-α-D-glucosaminyl bromide with a slight excess of 1-*O*-benzoyl-5-*O*-benzyl-2,3-*O*-isopropylidene-D-ribitol in the presence of silver carbonate gave, on removal of protecting groups, a mixture of 4-*O*-α- and 4-*O*-β-D-glucosaminyl-D-ribitol containing a high proportion of the α-anomer. Since few methods are available for the synthesis of α-D-glucosaminides a demonstration of the general applicability of this reaction would be of considerable interest. As both 2-*O*-α- and 2-*O*-β-D-glucosaminylglycerol were required in these laboratories as reference compounds the reaction of this glucosaminyl bromide with 1,3-di-*O*-benzylglycerol was selected for examination.

The Koenigs–Knorr reaction was carried out as before,[1] only a slight excess of the hydroxylic component being used. The product was deacetylated catalytically in methanol and then heated with dilute acid; chloroform was used to extract 4-methoxybenzaldehyde and di-*O*-benzylglycerol and then benzyl groups were removed from the acid-soluble material by hydrogenolysis. Fractionation of the mixture on Dowex 1 (OH^-) resin with water as the eluant [2] gave three glucosamine derivatives; glucosamine itself was known to be retained by the resin under these conditions.[1] The first two derivatives to be eluted, isolated as their crystalline hydrochlorides, were identified as 2-*O*-α- and 2-*O*-β-D-glucosaminylglycerol, respectively; each gave 2,5-anhydromannose (chitose) and glycerol on deamination with nitrous acid.[3,4] The third derivative was identified chromatographically as 1-*O*-D-glucosaminyl-D-glucosaminide.[1]

2-*O*-D-Glucosaminylglycerol was produced in 25% yield by this reaction, the ratio of α- to β-anomer in the mixture being 0·39 : 1, whereas 4-*O*-D-glucosaminyl-D-ribitol was obtained in 15% yield, the ratio of α- to β-anomer being 7·3 : 1, when 1,3-di-*O*-benzylglycerol was replaced by 1-*O*-benzoyl-5-*O*-benzyl-2,3-*O*-isopropylidene-D-ribitol. Thus the αβ-mixture of glycosides produced from the more reactive alcohol contains much less of the α-anomer. Since treatment of this glycosyl bromide with methyl or benzyl

[1] Hardy, Buchanan, and Baddiley, *J.*, 1963, 3360.
[2] Austin, Hardy, Buchanan, and Baddiley, *J.*, 1963, 5350.
[3] Foster, Martlew, and Stacey, *Chem. and Ind.*, 1953, 825.
[4] Baddiley, Buchanan, Rajbhandary, and Sanderson, *Biochem. J.*, 1962, **82**, 439.

alcohols is known to give the corresponding β-glucosaminides,[5] it seems that α-glucosaminides will only be formed when the halide reacts with secondary alcohols, particularly those in which the reactivity has been reduced by steric effects. An explanation of this behaviour has already been suggested.[1]

In another experiment 3,4,6-tri-*O*-acetyl-*N*-diphenoxyphosphinyl-α-D-glucosaminyl bromide was condensed with 1,3-di-*O*-benzylglycerol in the presence of mercuric cyanide in benzene. Treatment of the crystalline product with benzyl alcoholic ammonia caused deacetylation and replacement of phenyl groups with benzyl groups [5] giving a crystalline dibenzyl phosphoramidate which was converted by hydrogenolysis into 2-*O*-β-D-glucosaminylglycerol, characterised as its hydrochloride.

A very small amount of 2-*O*-D-glucosaminylglycerol has recently been isolated as a degradation product of the intracellular teichoic acid of *Staphylococcus aureus*.[6] However, none of this material is now available to allow a determination of the configuration of the glycosidic linkage by comparison with the synthetic glycosides.

Experimental

Infrared spectra were determined on potassium bromide discs. Dowex 1 resin (2% cross-linkages; 200—400 mesh; OH^- form) was used for chromatography.[2] Evaporations were carried out under reduced pressure at bath temperatures below 50°.

Paper Chromatography.—Whatman No. 1 or 4 paper was used. The following solvent systems were employed by descending irrigation: (A) butan-1-ol–ethanol–water–ammonia (*d* 0·88) (40 : 10 : 49 : 1); [7] (B) butan-1-ol–pyridine–water (6 : 4 : 3).[8] The periodate–Schiff [9] and ninhydrin [10] reagents were used as sprays where appropriate.

1,3-*Di*-O-*benzyl*-2-O-(3,4,6-*tri*-O-*acetyl*-N-*diphenoxyphosphinyl*-β-D-*glucosaminyl*)*glycerol.*—1,3-Di-*O*-benzylglycerol [11] (0·82 g.) was added to anhydrous benzene (30 ml.) together with mercuric cyanide (1·5 g.). Benzene (5 ml.) was distilled from the mixture before 3,4,6-tri-*O*-acetyl-*N*-diphenoxyphosphinyl-α-D-glucosaminyl bromide [5] (1·83 g., 1 mol.) was added. More benzene (3 ml.) was distilled from the mixture which was then heated under reflux for 3·5 hr. with vigorous stirring. After filtration, chloroform (60 ml.) was added to the filtrate which was washed twice with dilute sodium chloride solution and three times with water, dried (K_2CO_3), and evaporated to a white crystalline mass. This was washed with ether and crystallised from ethanol giving the β-D-*glucosaminide* (1·3 g., 55%), m. p. 146—147°, $[\alpha]_D$ −5° (*c* 3·6 in $CHCl_3$) (Found: C, 62·0; H, 5·9; N, 1·9; P, 4·2. $C_{41}H_{46}NO_{13}P$ requires: C, 62·2; H, 5·9; N, 1·8; P, 3·9%).

1,3-*Di*-O-*benzyl*-2-O-(N-*dibenzyloxyphosphinyl*-β-D-*glucosaminyl*)*glycerol.*—The above diphenyl phosphoramidate (0·55 g.) was stirred with benzyl alcohol (40 ml.) which had previously been saturated at 0° with dry ammonia. When all solid material had dissolved, the solution was allowed to warm to room temperature and left for 4 days. Ammonia was removed by evaporation at room temperature and then benzyl alcohol was removed by distillation at 100°/2 mm. The solid residue was washed with ether and recrystallised from butan-2-one to give 1,3-*di*-O-*benzyl*-2-O-(N-*dibenzyloxyphosphinyl*-β-D-*glucosaminyl*)*glycerol* (0·29 g., 60%), m. p. 140—141° (Found: C, 64·4; H, 6·5; N, 2·0. $C_{37}H_{44}NO_{10}P$ requires: C, 64·1; H, 6·35; N, 2·0%).

2-O-β-D-*Glucosaminylglycerol.*—The above dibenzyl phosphoramidate (0·24 g.) was hydrogenated for 6 hr. over palladium (from 0·4 g. of oxide) in methanol–water (19 : 1; 20 ml.). After removal of catalyst the solution was examined chromatographically in solvent A. Only one compound was detected with $R_{Glucosamine}$ 1·05 which reacted with the periodate–Schiff and ninhydrin reagents. Evaporation of the solution to dryness gave an amorphous solid which was dissolved in water (10 ml.), and Dowex 2 (CO_3^{2-}) resin (5 ml.) was added with stirring.

[5] Zervas and Konstas, *Chem. Ber.*, 1960, **93**, 435.
[6] Rajbhandary and Baddiley, *Biochem. J.*, 1963, **87**, 429.
[7] Hirst, Hough, and Jones, *J.*, 1949, 928.
[8] Jeanes, Wise, and Dimler, *Analyt. Chem.*, 1951, **23**, 415.
[9] Baddiley, Buchanan, Handschumacher, and Prescott, *J.*, 1956, 2818.
[10] Consden and Gordon, *Nature*, 1948, **162**, 180.
[11] Austin, Hardy, Buchanan, and Baddiley, *J.*, 1964, 2128.

After filtration, the aqueous solution was evaporated to dryness giving a white powder (0·10 g.) which was boiled with ethanol (7 ml.), water being added until most of the solid material had dissolved. Insoluble material was removed and a 0·5% solution of hydrogen chloride in ethanol (3·5 ml.) was added. 2-O-β-D-*Glucosaminylglycerol hydrochloride* soon crystallised as rosettes of fine needles (0·08 g.), m. p. 214° (decomp.), $[\alpha]_D$ −20° (*c* 1·4 in H_2O) (Found: C, 37·7; H, 7·0; N, 4·9. $C_9H_{19}NO_7$,HCl requires: C, 37·3; H, 7·0; N, 4·8%).

Synthesis of 2-O-α-D-*Glucosaminylglycerol and* 2-O-β-D-*Glucosaminylglycerol.*—1,3-Di-*O*-benzylglycerol (1·8 g.) was dissolved in anhydrous benzene (20 ml.); Drierite (L. Light and Co., regular grade; 10 g.) and silver carbonate [12] (3·6 g.) were added, and the mixture was shaken vigorously for 15 hr. in the dark. A solution of 3,4,6-tri-*O*-acetyl-*N*-4-methoxybenzylidene-α-D-glucosaminyl bromide [5] (1·25 g., 0·39 mol.) in benzene (12 ml.) was introduced during 30 min. with constant shaking in the dark. The shaking with occasional release of carbon dioxide was continued for 24 hr. Fresh silver carbonate (1 g.) was added and further addition of the glycosyl bromide (1·25 g., 0·39 mol.) in benzene (12 ml.) was followed by shaking for 54 hr. The mixture was centrifuged and the supernatant layer and benzene washings were extracted twice with water, dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in methanol (60 ml.), sodium methoxide (from 0·034 g. of sodium) in methanol (17 ml.) added, and the mixture kept overnight. After neutralisation with solid carbon dioxide, the solution was evaporated to a syrup which was then boiled with 0·13N-sulphuric acid (100 ml.) for 40 min. and cooled. N-Sulphuric acid (5 ml.) was then added and the mixture was extracted four times with chloroform, neutralised with calcium carbonate, filtered, and evaporated to dryness. The residual syrup was shaken with 2 : 3 v/v aqueous ethanol (70 ml.) and filtered. The filtrate was acidified to pH 5 and hydrogenated with palladium (from 1·0 g. of oxide) at atmospheric pressure for 40 hr. After removal of catalyst the solution was evaporated to a red-brown syrup (1·25 g.).

This material was dissolved in a little water and applied to a column of Dowex 1 (OH^-) resin (60 ml.).[2] Elution was carried out with water; the first 60 ml. of eluate was discarded and then fractions (10 ml.) were collected. Examination of these in solvent A showed that the product had been resolved into three components. Fractions 16—20 contained a compound which had the same $R_{Ribitol}$ (0·25) as 1-*O*-α-D-glucosaminyl-β-D-glucosaminide; [1] this material was not isolated. Fractions 7—14 contained a compound with the same $R_{Ribitol}$ (0·80) as 2-*O*-β-D-glucosaminylglycerol. These fractions were united and evaporated to dryness giving the glycoside as an amorphous solid (0·25 g.); its hydrochloride, prepared as described above, was identified by comparison of the m. p., specific rotation, and infrared spectrum with those of an authentic specimen.

2-O-α-D-*Glucosaminylglycerol.*—Fractions 3—6 contained a compound with $R_{Ribitol}$ 0·75 in solvent A. The fractions were united and evaporated to an amorphous solid (0·82 g.). This material was boiled with a mixture of ethanol (5 ml.) and methanol (0·5 ml.). Insoluble material was removed and a 0·5% solution of hydrogen chloride in ethanol (2·7 ml.) was added. On adding ether and cooling, crystallisation occurred giving microcrystalline 2-O-α-D-*glucosaminylglycerol hydrochloride*, m. p. 160—162°, $[\alpha]_D$ +117° (*c* 1·3 in H_2O) (Found: C, 37·5; H, 7·2; N, 5·0. $C_9H_{19}NO_7$,HCl requires: C, 37·3; H, 7·0; N, 4·8%).

Action of Sodium Nitrite on 2-O-D-*Glucosaminylglycerol Hydrochloride.*—A sample of each anomer (0·003 g.) was dissolved in water (1 ml.) and sodium nitrite (0·03 g.) was added. Each solution was heated at 70° for 5 hr. and then passed through a small column of Dowex 50 (NH_4^+) resin; the eluate was heated at 100° for 10 min., evaporated to dryness, and examined in solvent B. The products, in each case, were glycerol (R_F 0·57) and 2,5-anhydromannose (R_F 0·60).

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Department of Organic Chemistry,
University of Newcastle upon Tyne. [*Received, February 17th*, 1964.]

[12] Wolfrom, Pittet, and Gillam, *Proc. Nat. Acad. Sci. U.S.A.*, 1961, **47**, 700.