623. *Synthesis of* 4-O-β-D-*Glucosaminyl*-D-*ribitol and* 4-O-α-D-*Glucosaminyl*-D-*ribitol, Degradation Products of the Ribitol Teichoic Acid from* Staphylococcus aureus *H.*

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Both 4-*O*-β- and 4-*O*-α-D-glucosaminyl-D-ribitol, degradation products of the teichoic acid from *Staphylococcus aureus* H walls, have been synthesised by a Koenigs–Knorr reaction from 3,4,6-tri-*O*-acetyl-*N*-4-methoxybenzylidene-α-D-glucosaminyl bromide and 1-*O*-benzoyl-5-*O*-benzyl-2,3-*O*-isopropylidene-D-ribitol. The first reported isolation of the α-anomer from the αβ-mixture of glucosaminides obtained from the teichoic acid is described, and evidence is presented that no anomerisation of fully acetylated glucosaminides occurs under alkaline conditions.

Teichoic acids [1] have been isolated from the walls and cell contents of a number of Gram-positive bacteria (for a review see ref. 2). When the wall teichoic acid from *Staphylococcus aureus* H was hydrolysed with alkali and then treated with a phosphomonoesterase a phosphorus-free compound was produced which had the chromatographic properties of an *O*-glucosaminylribitol.[1] When mild alkali was used an *O*-(*N*-acetylglucosaminyl)ribitol was produced. This compound appeared to be a mixture of 4-*O*-(*N*-acetyl-β-D-glucosaminyl)-D-ribitol and the corresponding α-anomer.[3-5] The β-anomer, the major component, was isolated as the crystalline octa-acetate but the α-anomer was not isolated.

A synthesis of the anomeric pair of 4-*O*-D-glucosaminyl-D-ribitols was obviously desirable. Moreover, in view of suggestions [6,7] that alkali may cause β→α isomerisation of *N*-acetylglucosaminides it was necessary to establish whether the α-anomer was an artefact of degradation of the teichoic acid.

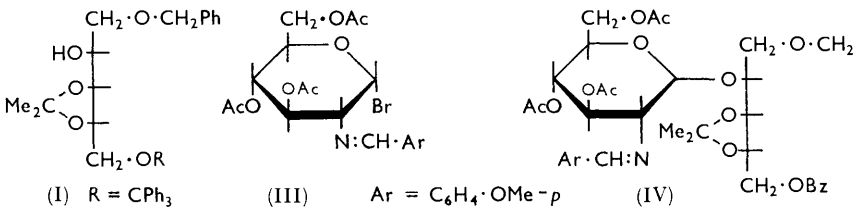
A synthesis of the β-glucosaminide involving a Koenigs–Knorr reaction between a ribitol derivative having only the 4-hydroxyl (D-form) free and a suitably protected

[1] Armstrong, Baddiley, Buchanan, Carss, and Greenberg, *J.*, 1958, 4344.
[2] Baddiley, *J. Roy. Inst. Chem.*, 1962, **86**, 366.
[3] Baddiley, Buchanan, Hardy, Martin, RajBhandary, and Sanderson, *Biochim. Biophys. Acta*, 1961, **52**, 406.
[4] Baddiley, Buchanan, RajBhandary, and Sanderson, *Biochem. J.*, 1962, **82**, 439.
[5] Baddiley, Buchanan, Martin, and RajBhandary, *Biochem. J.*, 1962, **85**, 49.
[6] Hough and Taha, *J.*, 1956, 2042.
[7] Leaback and Walker, *J.*, 1957, 4754.

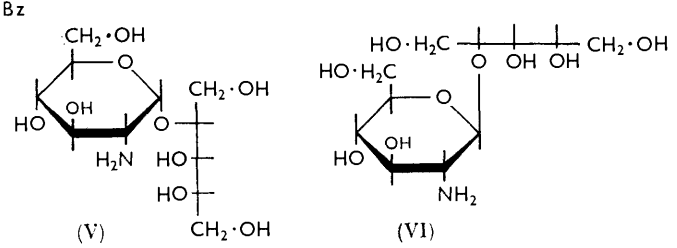
α-D-glucosaminyl bromide was envisaged. Two suitably protected ribitol derivatives were already available: (a) 5-*O*-benzyl-2,3-*O*-isopropylidene-1-*O*-triphenylmethyl-D-ribitol (I) which has been used in a synthesis of 4-*O*-β-D-glucopyranosyl-D-ribitol,[8] and (b) 1-*O*-benzoyl-5-*O*-benzyl-2,3-*O*-isopropylidene-D-ribitol (II) which has been used in a synthesis of 4-*O*-α-D-glucopyranosyl-D-ribitol.[9] The latter was preferred since it is the more stable in the Koenigs–Knorr reaction; the triphenylmethyl residue in the former compound is partly removed under these conditions.[8]

The choice of a suitable glycosyl halide was more difficult. Although Micheel and Petersen [10] crystallised *N*-acetyl-3,4,6-tri-*O*-acetyl-α-D-glucosaminyl bromide all previous attempts to isolate it had failed since, in the presence of traces of water, it readily rearranges to 1,3,4,6-tetra-*O*-acetyl-α-D-glucosamine hydrobromide.[7,11,12] Some simple glucosaminides have been made by using fresh chloroform solutions of this glycosyl bromide in an attempt to reduce acyl migration.[7,12] The more stable *N*-acetyl-3,4,6-tri-*O*-acetyl-α-D-glucosaminyl chloride has also been used for preparing glucosaminides [7,12] but it is probably insufficiently reactive for the purpose required here. Several more convenient methods for the synthesis of glucosaminides, free from the disadvantage of acyl migration, are now available. Lloyd and Stacey [13] found that 3,4,6-tri-*O*-acetyl-*N*-2,4-dinitrophenyl-α-D-glucosaminyl bromide gave good yields of glucosaminides, and other workers have shown that 3,4,6-tri-*O*-acetyl-α-D-glucosaminyl bromides with the amino-group in the form of the 4-methoxybenzylidene derivative,[14] or as the diphenyl phosphoramidate [14] or toluene-ω-sulphonamide,[15] are also useful in this field.

The glycosyl halide used in the present work was 3,4,6-tri-*O*-acetyl-*N*-4-methoxybenzylidene-α-D-glucosaminyl bromide (III). Zervas and Konstas used it to prepare methyl and benzyl β-glucosaminides in good yield by condensation with the corresponding alcohol in the presence of silver carbonate and anhydrous calcium sulphate. Condensation of this halide with the protected ribitol derivative (II) was carried out in benzene under



(I) R = CPh_3 (III) Ar = C_6H_4·OMe-*p* (IV)
(II) R = Bz



(V) (VI)

similar conditions. The product (IV) was deacylated catalytically in methanol, then heated with dilute acid to remove the methoxybenzylidene and isopropylidene groups, and the benzyl group was removed by hydrogenolysis. Paper chromatography in an

[8] Baddiley, Buchanan, and Hardy, *J.*, 1961, 2180.
[9] Sargent, Buchanan, and Baddiley, *J.*, 1962, 2184.
[10] Micheel and Petersen, *Chem. Ber.*, 1959, **92**, 298.
[11] Micheel, Van der Kamp, and Wulff, *Chem. Ber.*, 1955, **88**, 2011.
[12] Inouye, Onodera, Kitaoka, and Ochiai, *J. Amer. Chem. Soc.*, 1957, **79**, 4218.
[13] Lloyd and Stacey, *Chem. and Ind.*, 1955, 917; *Tetrahedron*, 1960, **9**, 116.
[14] Zervas and Konstas, *Chem. Ber.*, 1960, **93**, 435.
[15] Onodera, Kitaoka, and Ochiai, *J. Org. Chem.*, 1962, **27**, 156.

ammoniacal solvent (A) indicated the presence of ribitol, glucosamine, and two glucosamine derivatives, one of which had the chromatographic properties of the required glucosaminide. Glucosamine was removed with an excess of Dowex 1 (OH^-) resin, a method also useful for removing hexoses from Koenigs–Knorr reaction products (P. W. Austin, unpublished). The mixture was fractionated on Dowex 50 (H^+) resin, ribitol was eluted with water, and the two glucosamine derivatives were eluted with ammonia; these were separated by further chromatography on this resin, when the ribitol glucosaminide was eluted with 0·3N-hydrochloric acid;[16] the other glucosamine derivative was more strongly adsorbed, being eventually eluted with dilute ammonia.

The paper-chromatographic behaviour of this second compound was that expected of a diglucosaminide. Deamination with nitrous acid gave only 2,5-anhydromannose (chitose),[17] and the compound is probably a 1-*O*-D-glucosaminyl-D-glucosaminide (cf. ref. 12).

Deamination of the ribitol glucosaminide with nitrous acid gave chitose and ribitol;[4] the yield of this glucosaminide from the glycosyl bromide (III) was approximately 15%. Paper chromatography in propan-2-ol–hydrochloric acid (solvent B) showed that it was a mixture of two components; the minor component had the same R_F value as the required β-glucosaminide and gave an identical colour with the periodate–Schiff spray reagents,[18] whereas the major component had a greater R_F value and gave a different colour. The specific rotation of the mixture of glucosaminides, $[\alpha]_D$ +100°, indicated that the majority of glycosidic linkages had the α-configuration.

The two isomers were separated by careful chromatography on Dowex 50 (H^+) resin with 0·26N-hydrochloric acid as the eluant. The α-glucosaminide was eluted after the β-anomer and was isolated as its crystalline hydrochloride. In order to establish whether this was the hydrochloride of 4-*O*-α-D-glucosaminyl-D-ribitol (V), which was known to be present in preparations of ribitol glucosaminide from *S. aureus* teichoic acid[4,5] but had never been isolated, a sample of the glucosaminide mixture from the teichoic acid was chromatographed on Dowex resin as described above. One component was obtained as its crystalline hydrochloride and shown to be identical with the synthetic hydrochloride by comparison of m. p.s and behaviour on paper chromatograms.

The synthetic β-anomer gave an octa-acetate which crystallised from ether. The infrared spectrum and m. p. of this material were different from those of the octa-acetate of 4-*O*-β-D-glucosaminyl-D-ribitol (VI) which had been obtained from the teichoic acid and crystallised from methanol–water.[4] However, when the latter acetate was crystallised from ether it showed an infrared spectrum and m. p. identical with those of the synthetic acetate, and paper chromatography of a mixture of the two revealed only one component. The crystals from methanol–water are those of a monohydrate,[4] and presumably those from ether are an anhydrous form of this acetate.

In the α,β-mixture of ribitol glucosaminides produced by this Koenigs–Knorr reaction there was about 88% of the α-isomer (V), whilst there was approximately 15% of this compound in the α,β-mixture of ribitol glucosaminides from the *S. aureus* teichoic acid. This particular sample[4,5] of teichoic acid had $[\alpha]_D$ +6°; however, the specific rotation of samples of teichoic acid prepared from different batches of *S. aureus* walls varied, and one sample with $[\alpha]_D$ −15·6° did not contain detectable amounts of α-linkages.[4,5]

The discovery that 3,4,6-tri-*O*-acetyl-*N*-4-methoxybenzylidene-α-D-glucosaminyl bromide (III) gives a high proportion of an α-glucosaminide under the reaction conditions described is of considerable interest. It may be significant that in the syntheses of methyl and benzyl β-D-glucosaminides described by Zervas and Konstas[14] this glycosyl bromide was treated with solvent quantities of methyl and benzyl alcohol and gave high yields in a short (3 hr.) time. In the present reaction only 1·3 mol. of the alcohol (II) were used and a relatively low yield of glucosaminide was obtained even after a long time (80 hr.).

[16] Crumpton, *Biochem. J.*, 1959, **72**, 479.
[17] Foster, Martlew, and Stacey, *Chem. and Ind.*, 1953, 825.
[18] Baddiley, Buchanan, Handschumacher, and Prescott, *J.*, 1956, 2818.

In the former cases replacement of bromine occurs with inversion, whereas in the latter case the slower overall reaction permits more complete separation of bromide ion and glycosyl cation, thus favouring the formation of an α-glucosaminide. In the usual *O*-acylglucosyl or *N*-acylglucosaminyl halides the 2-*O*- or *N*-acyl group also hinders the formation of α-glycosidic linkages,[19] whereas the *N*-4-methoxybenzylidene group would not be expected to show this neighbouring-group effect at C_1; the general application of this observation is under investigation. It is perhaps relevant that 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucose (Brigl's anhydride) [20] reacts with alcohols in two ways. With simple alcohols (ROH) it gives good yields of β-glucosides,[20,21] but when the group R is more bulky the reaction is slow and a high proportion of α-glycosidic linkages is formed; this principle was used recently in a synthesis of 4-*O*-α-D-glucopyranosyl-D-ribitol.[9] Another example of the formation of α-glucosaminides in a Koenigs–Knorr reaction was reported recently by Lloyd *et al.*;[13,22] they found that in the presence of pyridine 3,4,6-tri-*O*-acetyl-*N*-2,4-dinitrophenyl-α-D-glucosaminyl bromide reacted with alcohols to give mixtures of α- and β-glucosaminides containing high proportions of the α-anomers.

The effect of alkali on acetylated β-glucosaminides has been studied in view of the suggestion [6,7] that partial inversion of configuration of the glycosidic linkage can occur during deacetylation of these compounds under alkaline conditions. If this were correct, the isolation of both the α- and β-glucosaminides and their *N*-acetyl derivatives by alkali hydrolysis of a teichoic acid would not necessarily imply the presence of both types of linkage in the teichoic acid polymer. Initially experiments were carried out on methyl *N*-acetyl-3,4,6-tri-*O*-acetyl-β-D-glucosaminide. That this compound was free from traces of the α-anomer was demonstrated by paper chromatography with the dimethyl sulphoxide–di-isopropyl ether solvent system and silver nitrate–sodium hydroxide reagents developed by Wickberg.[23] It is interesting that the ratio of the R_F value of the β-anomer to that of the α-anomer is 1 : 3·5, representing a remarkable separation for such closely similar compounds. De-*O*-acetylation of the β-anomer was carried out by using methanolic ammonia, and paper chromatography showed methyl *N*-acetyl-β-D-glucosaminide as the only product. This was further treated with barium hydroxide under the conditions used in the alkali degradation of *S. aureus* teichoic acid.[4] The product was re-acetylated and examined chromatographically; no α-anomer was detected.

A similar experiment was carried out on the octa-acetate of 4-*O*-β-D-glucosaminyl-D-ribitol (VI). The purity of this acetate was also demonstrated by using Wickberg's techniques; the ratio of the R_F value of this compound to that of the corresponding α-anomer was also 1 : 3·5. The alkali hydrolysis involved successive treatment with methanolic ammonia, Dowex 1 (OH^-) resin, and barium hydroxide. Re-acetylation and paper chromatography showed the absence of any α-anomer. Comparable results have been obtained by Kuhn and his co-workers [24] who used *O*-acetylation, fractional crystallisation of the tetra-acetate, and de-*O*-acetylation under alkaline conditions to purify samples of methyl *N*-acetyl-α- and -β-glucosaminides. It must be assumed, therefore, that the *N*-acetyl-α-glucosaminides which have been reported [6,7] as products of de-*O*-acetylation of acetylated β-glucosaminides were produced from the corresponding acetylated α-glucosaminides with which the β-anomers were contaminated.

Experimental

Infrared spectra were determined on potassium bromide discs. Neutral alumina (grade O alumina neutralised with acetic acid and re-activated), and Dowex 50 W resin (8% cross-linkages; 200—400 mesh; H^+ form) which had been washed thoroughly with 4N-hydrochloric

[19] Haynes and Newth, *Adv. Carbohydrate Chem.*, 1955, **10**, 207.
[20] Brigl, *Z. physiol. Chem.*, 1922, **122**, 245.
[21] Hickinbottom, *J.*, 1928, 3140.
[22] Lloyd and Roberts, *Proc. Chem. Soc.*, 1960, 250.
[23] Wickberg, *Acta Chem. Scand.*, 1958, **12**, 615.
[24] Kuhn, Zilliken, and Gauhe, *Chem. Ber.*, 1953, **86**, 466.

acid, were used for chromatography. Evaporations were carried out under reduced pressure at bath temperatures below 50°.

Paper Chromatography.—Whatman No. 1 or No. 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);[25] (B) propan-2-ol–concentrated hydrochloric acid–water (65 : 17 : 18);[26] (C) butan-1-ol–ethanol–water (40 : 11 : 19);[6] (D) butan-1-ol–pyridine–water (6 : 4 : 3);[27] (E) di-isopropyl ether on paper treated with dimethyl sulphoxide.[23] The periodate–Schiff,[18] silver nitrate–sodium hydroxide,[23] ninhydrin,[28] and aniline phthalate [29] reagents were used as sprays where appropriate.

Synthesis of 4-O-β-D-*Glucosaminyl*-D-*ribitol and* 4-O-α-D-*Glucosaminyl*-D-*ribitol.*—1-*O*-Benzoyl-5-*O*-benzyl-2,3-*O*-isopropylidene-D-ribitol [9] (1·22 g.) was dissolved in pure dry benzene (14 c.c.); Drierite (L. Light & Co., regular grade; 4·5 g.) and silver carbonate [30] (1·6 g.) were added and the mixture was shaken vigorously for 12 hr. in the dark. A solution of 3,4,6-tri-*O*-acetyl-*N*-4-methoxybenzylidene-α-D-glucosaminyl bromide [14] (0·6 g., 0·4 mol.) in benzene (4 c.c.) was introduced during 30 min. with constant shaking in the dark. The shaking, with occasional release of carbon dioxide, was continued for 24 hr. Further addition of the glycosyl bromide (0·53 g., 0·37 mol.) in benzene (5 c.c.) was followed by shaking for 12 hr.; fresh silver carbonate (0·5 g.) was added and the shaking continued for a further 48 hr. The mixture was centrifuged and the supernatant layer and the benzene washings were extracted twice with water and evaporated to dryness. The residue was dissolved in methanol (30 c.c.), sodium methoxide (from 0·04 g. of sodium) in methanol (15 c.c.) added, and the mixture kept overnight. After neutralisation with solid carbon dioxide, the solution was evaporated to dryness, giving a syrup (2 g.) which was boiled with 0·13N-sulphuric acid (55 c.c.) for 40 min. After cooling, the mixture was extracted three times with chloroform, neutralised with barium carbonate, filtered, and evaporated to a syrup (1·1 g.). This was shaken with 2 : 3 v/v aqueous ethanol (30 c.c.) and filtered. The filtrate was acidified to pH 4 and hydrogenated with palladium (from 1·2 g. of oxide) at atmospheric pressure for 40 hr. After removal of catalyst the solution was evaporated to dryness. Paper chromatography of the product in solvent A showed a mixture of four components: ribitol, glucosamine, and two compounds which reacted with the ninhydrin and periodate–Schiff reagents but not with aniline phthalate. The faster-moving of these had the same R_{ribitol} (0·40) as the ribitol glucosaminide from teichoic acid; the slower-moving compound had R_{ribitol} 0·27.

The mixture was dissolved in water (50 c.c.), and Dowex 1 (OH^-) resin (50 c.c.) was added. After being shaken for 65 hr. at 28° the mixture was applied to a short column of Dowex 1 (OH^-) resin. When the aqueous solution had passed through the resin, the column was washed with water (400 c.c.), and the eluate and washings were evaporated to a syrup (0·61 g.); chromatography in solvent A showed that glucosamine was no longer present. The syrup was dissolved in a little water and applied to a column of Dowex 50 (H^+) resin (60 c.c.). Ribitol (0·4 g.) was eluted with water and recovered by evaporation; subsequent passage of 0·3N-hydrochloric acid (250 c.c.) and neutralisation of the eluate with Dowex 1 (CO_3^{2-}) resin, filtration, and evaporation gave a syrup (0·11 g.) which was shown by chromatography in solvent A to be the compound with the same R_{ribitol} as 4-*O*-β-D-glucosaminyl-D-ribitol. The column was washed with water until the washings were neutral and the other glucosaminide (0·04 g.), R_{ribitol} 0·27 in solvent A, was eluted with N-ammonia solution. When the first glucosaminide, $[\alpha]_D^{24}$ +100° (*c* 3·0 in H_2O), was chromatographed in solvent B two components were observed: the minor component, had the same R_F (0·42) as 4-*O*-β-D-glucosaminyl-D-ribitol, while the other had R_F 0·46. These two components were separated on Dowex 50 (H^+) resin (50 c.c.). The resin was washed with 0·26N-hydrochloric acid (250 c.c.), and the mixture, dissolved in a small volume of this solution, was applied to the column. Elution was carried out with acid of the same strength; fractions (10 c.c.) were collected. These were neutralised with Dowex 1 (CO_3^{2-}) resin, filtered, and evaporated to dryness. Chromatography in solvent B showed that the glucosaminide with R_F 0·42 (0·012 g.) was eluted from the resin before the glucosaminide with R_F 0·46 (0·09 g.); both were chromatographically pure syrups.

[25] Hirst, Hough, and Jones, *J.*, 1949, 928.
[26] Smith and Wyatt, *Biochem. J.*, 1951, **49**, 144.
[27] Jeanes, Wise, and Dimler, *Analyt. Chem.*, 1951, **23**, 415.
[28] Consden and Gordon, *Nature*, 1948, **162**, 180.
[29] Partridge, *Nature*, 1949, **164**, 443.
[30] Wolfrom, Pittet, and Gillam, *Proc. Nat. Acad. Sci. U.S.A.*, 1961, **47**, 700.

4-O-β-D-*Glucosaminyl*-D-*ribitol* (*Octa-acetate*).—The material with R_F 0·42 in solvent B was treated with acetic anhydride (0·3 c.c.) in anhydrous pyridine (1·5 c.c.) for 2 days at room temperature. The acetate was isolated by means of chloroform; it was purified by chromatography on neutral alumina and formed crystals, m. p. 126°, from ether. The octa-acetate of 4-*O*-β-D-glucosaminyl-D-ribitol from the teichoic acid,[4] previously crystallised from methanol–water as a monohydrate, m. p. 137—138°, gave an infrared spectrum different from that given by this acetate. However, when ether rather than methanol–water was used for crystallisation, a second crystalline form of the acetate, m. p. 127—128°, was obtained. These crystals gave an infrared spectrum identical with that of the acetate of the synthetic glucosaminide, and a mixture of the two compounds had m. p. 126—127° and gave only one spot, $R_{\text{penta-}O\text{-acetyl-}\beta\text{-D-glucose}}$ 0·07, when chromatographed in solvent E.

4-O-α-D-*Glucosaminyl*-D-*ribitol* (*Hydrochloride*).—The glucosaminide (0·09 g.) with R_F 0·46 in solvent B was dissolved in boiling ethanol containing a trace of water. Insoluble material was removed by centrifugation. A 0·5% solution of hydrogen chloride (1·3 mol.) in anhydrous ethanol was added and the solution was cooled. Crystallisation occurred overnight, giving material (0·053 g.) with m. p. 193—194°, $[\alpha]_D^{22}$ +110° (*c* 0·3 in H_2O) (Found: C, 37·7; H, 6·7. $C_{11}H_{23}NO_9$,HCl requires C, 37·8; H, 6·9%). Addition of ether to the mother-liquors gave a second batch of crystals (0·018 g.).

A sample of the hydrochloride (0·02 g.) was acetylated in the same way as the β-anomer, giving a syrupy octa-acetate (Found: C, 49·6; H, 6·1. $C_{27}H_{39}NO_{17}$ requires C, 49·9; H, 6·0%). It was homogeneous in solvent E ($R_{\text{penta-}O\text{-acetyl-}\beta\text{-D-glucose}}$ 0·25). This acetate was de-*O*-acetylated in methanol (15 c.c., previously saturated at 0° with ammonia) during 36 hr. at room temperature. After evaporation the residue was chromatographed on a carbon–Celite column (2 g. of Norit A, and 1 g. of Celite 545). After washing of the column with a large volume of 1% ethanol, the amorphous *N*-acetylglucosaminide was eluted with 15% ethanol. It was homogeneous in solvent A; the ratio of its R_F value to that of 4-*O*-(*N*-acetyl-β-D-glucosaminyl)-D-ribitol was 1·1 : 1.

A sample of the *N*-acetylglucosaminide was hydrolysed with 2N-hydrochloric acid at 100° for 18 hr. The hydrolysate was passed through a small column of Dowex 3 (OH^-) resin and evaporated to dryness. Examination in solvent A showed that the products were glucosamine and anhydroribitol (R_{ribitol} 1·66), together with a little ribitol and ribitol glucosaminide (cf. ref. 4). A separation of the four anhydropentitols is possible in this solvent since their R_{ribitol} values are: anhydroribitol, 1·66; anhydrolyxitol, 1·70; anhydroxylitol, 1·88; anhydroarabitol, 1·95.

Action of Nitrous Acid.—The α-glucosaminylribitol hydrochloride (0·003 g.) and sodium nitrite (0·03 g.) were dissolved in water (1 c.c.), and the solution was heated at 65° for 5 hr. It was then passed through a small column of Dowex 50 (NH_4^+) resin, and the eluate was heated at 80° for 15 min., evaporated to dryness, and examined in solvent D. The products were ribitol (R_F 0·37) and 2,5-anhydromannose (R_F 0·58) (cf. ref. 4).

The glucosamine derivative with R_{ribitol} 0·27 in solvent A produced in the Koenigs–Knorr reaction was not examined closely. It gave a hygroscopic hydrochloride, $[\alpha]_D^{23}$ +90° (*c* 0·3 in H_2O), with R_F 0·19 in solvent B. On treatment with nitrous acid as described above it gave only 2,5-anhydromannose and so was probably 1-*O*-α-D-glucosaminyl-β-D-glucosaminide.

4-O-α-D-*Glucosaminyl*-D-*ribitol from Teichoic Acid.*—The teichoic acid (0·3 g.) with $[\alpha]_D$ +6° had previously been degraded by treatment with saturated (room temperature) barium hydroxide solution at 100° for 30 min., followed by incubation with a phosphomonoesterase; α,β-mixtures of anomers of 4-*O*-D-glucosaminyl-D-ribitol (0·025 g.) and of the corresponding *N*-acetyl derivative (0·096 g.) had been produced, but in the present work only the α,β-mixture of ribitol glucosaminides was examined; only the β-anomer was visible on chromatography in solvent B. The mixture was resolved on a column of Dowex 50 (H^+) resin (18 c.c.) prepared in 0·26N-hydrochloric acid by a procedure similar to that described for the separation of the synthetic glucosaminides. The products were the previously described β-glucosaminide (0·015 g.) and chromatographically pure 4-*O*-α-D-glucosaminyl-D-ribitol (0·0025 g.). The latter compound in solvent B gave the same colour with the periodate–Schiff reagents as did the synthetic α-glucosaminylribitol, and a mixture of the two ran as a single spot. When it was dissolved in hot ethanol and treated with ethanolic hydrogen chloride a crystalline hydrochloride was obtained with m. p. 190°, undepressed on admixture with the synthetic α-glucosaminide hydrochloride.

Reaction of Acetylated β-Glucosaminides with Alkaline Reagents.—(1) *Tetra-acetate of methyl β-D-glucosaminide.* Methyl *N*-benzyloxycarbonyl-β-D-glucosaminide was prepared by the method described by Foster *et al.*[31] Hydrogenolysis [32] and acetylation with acetic anhydride in pyridine gave the methyl β-D-glucosaminide tetra-acetate which, after recrystallisation twice from ethanol, had m. p. 160—161°, $[\alpha]_D^{24}$ −26° (*c* 2·0 in chloroform) (Hough and Taha [6] give m. p. 160°, $[\alpha]_D$ −21°). Chromatography in solvent E showed that this material ($R_{\text{penta-}O\text{-acetyl-}\beta\text{-D-glucose}}$ 0·05) was free from traces of the corresponding α-anomer ($R_{\text{penta-}O\text{-acetyl-}\beta\text{-D-glucose}}$ 0·175).

The pure β-anomer (0·025 g.) was dissolved in anhydrous methanol (20 c.c.), and the solution saturated at 0° with ammonia. The reaction mixture was allowed to reach room temperature slowly and was then kept for 3 days. Evaporation and examination in solvent C with the silver nitrate–sodium hydroxide reagents revealed the presence of methyl *N*-acetyl-β-D-glucosaminide, but no trace of the α-anomer was detected. The ratio of the R_F value of the α-anomer to that of the β-anomer was 1·14 : 1; the R_F values quoted by Hough and Taha [6] are the reverse of those given here. The product of de-*O*-acetylation was dissolved in saturated (room temperature) barium hydroxide solution (5 c.c.), and the solution was boiled for 30 min. It was then passed through Dowex 50 (NH_4^+) resin (7 c.c.), and the eluate was evaporated to dryness. This partially de-*N*-acetylated product was acetylated with acetic anhydride in pyridine, and the acetate was examined in solvent E. Only the tetra-acetate of methyl β-D-glucosaminide was observed.

(2) *Octa-acetate of 4-O-β-D-glucosaminyl-D-ribitol.* The chromatographically pure acetate (0·007 g.) was treated with methanolic ammonia as described above. The product was dissolved in water (4 c.c.), Dowex 1 (OH^-) resin (3 c.c.) was added, and the mixture was shaken for 60 hr. at 28°. The resin was filtered off and thoroughly washed, and filtrate and washings were evaporated to dryness. The residue was dissolved in saturated (room temperature) barium hydroxide solution and heated at 100° for 5 hr. The solution was passed through Dowex 50 (NH_4^+) resin and evaporated to dryness. The product was acetylated with acetic anhydride in pyridine, and the octa-acetate examined in solvent E; only the octa-acetate of 4-*O*-β-D-glucosaminyl-D-ribitol was observed.

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Newcastle upon Tyne, 1. [*Received, December 28th,* 1962.]

[31] Foster, Horton, and Stacey, *J.*, 1957, 81.
[32] Neuberger and Rivers, *J.*, 1939, 122.