93. *Studies in the Amino-Sugars. Part II. The Action of Dilute Alkali Solution on* N-*Acylglucosamines.*

By Theodore White.

N-Acylglucosamines, after treatment with hot dilute alkali solution, give a red-purple coloration with Ehrlich's reagent. It has been suggested that this is due to the presence, in the reaction mixture, of heterocyclic derivatives formed by elimination of a molecule of water and consequent linkage of the *N*-acyl chain with the reducing group of the amino-sugar derivative concerned. The author has investigated the reaction and it is concluded that, under the influence of hot dilute alkali solution, *N*-acetylglucosamine forms a glucoxazoline, whereas *N*-α-bromopropionylglucosamine forms a glucoxazine.

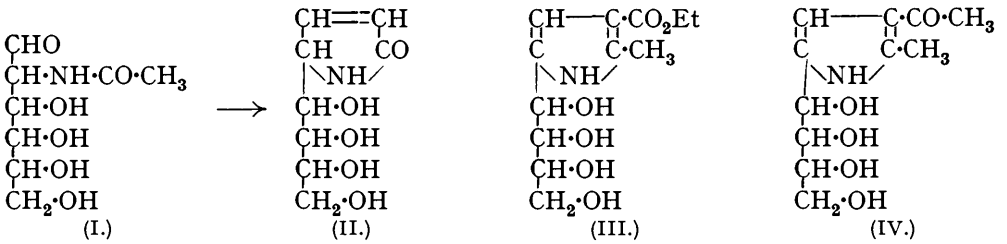
Early postulates regarding glucopeptides led to much work on *N*-acylglucosamines with a view to applying the knowledge so gained to investigations on glucopeptides of this sugar. *N*-Acetylglucosamine was first prepared by Breuer (*Ber.*, 1898, **31**, 2193) and a convenient method of preparation was described by Zuckerkandl and Messiner-Klebermass (*Biochem. Z.*, 1931, **236**, 19). The *N*-α-halogenoacylglucosamines were discussed by

Weizmann and Hopwood (*Proc. Roy. Soc.*, 1913, *A*, **88**, 433) and by Bertho, Holder, Meiser, and Huther (*Annalen*, 1931, **485**, 127). Irvine and Earl (J., 1922, **121**, 2376) condensed glucosamine with salicylaldehyde to form an anil, and Bergmann and Zervas (*Ber.*, 1931, **64**, 975) utilised the corresponding anisylideneglucosamine for the synthesis of *N*-acylglucosamines and glucopeptides of glucosamine. The latter, like some of the earlier workers, prepared *N*-α-halogenoacylglucosamines with a view to their conversion into glucopeptides of glucosamine. The results were complicated by the extraordinary capacity of glucosamine and its *N*-acyl derivatives for internal interaction between the hydroxylic reducing group and the amino-group in the free sugar, or the *N*-acyl side chain in the latter derivatives. This, in some cases, led to the formation of heterocyclic amino-sugar derivatives.

The postulate that glucosamine derivatives could, by intermolecular interaction, give rise to heterocyclic derivatives was first propounded by Irvine and Hynd (J., 1912, **101**, 1128; 1913, **103**, 41; 1914, **105**, 698), who, while investigating various glucosaminides, observed them to display an abnormal resistance to acid hydrolysis. It was suggested in this and later work by Irvine and Fyfe (J., 1914, **105**, 1642) and Hynd and McFarlane (*Biochem. J.*, 1926, **20**, 1264) that free glucosamine and glucosaminides have a betaine structure, but recent work by Moggridge and Neuberger (J., 1938, 745) indicated that the concept of the existence of a four-membered heterocyclic ring is not in this case justified.

Weizmann and Hopwood (*loc. cit.*) attempted to form glucopeptides by ammonia treatment of *N*-α-bromoacylglucosamines, but obtained products which were indicated by Bertho, Holder, Meiser, and Huther (*loc. cit.*) to have heterocyclic ring formulations resulting from intermolecular reaction brought about by the reagent.

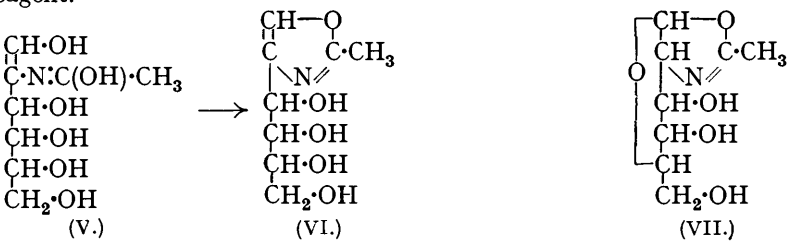
Muller (*Z. Biol.*, 1901, **42**, 564), after heating penta-acetyl glucosamine with dilute potassium hydroxide solution, found that an intense red-purple colour was produced on the addition of Ehrlich's reagent. Zuckerkandl and Messiner-Klebermass (*loc. cit.*) utilised this observation for the estimation of glucosamine, and suggested that, when *N*-acetylglucosamine is heated with dilute alkali solution, the aldehyde group reacts with the *N*-acetyl side chain (I) to form the pyrroline derivative (II), which, with Ehrlich's reagent, gives the colour characteristic of pyrroles.



(I.) (II.) (III.) (IV.)

Pauly and Ludwig (*Z. physiol. Chem.*, 1922, **121**, 170) had obtained the pyrrole derivatives of glucosamine (III and IV) by a different route and both these products gave a positive Ehrlich test.

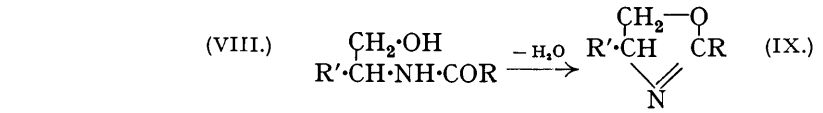
Morgan and Elson (*Biochem. J.*, 1934, **28**, 998) and Morgan (*ibid.*, 1936, **30**, 909), discussing the estimation of *N*-acetylglucosamine, suggested that alkali caused the sugar to react in the enolic form (V) and to be converted, by elimination of water, into the glucoxazole (VI) or the glucoxazoline (VII), the product giving rise to the colour with Ehrlich's reagent.



(V.) (VI.) (VII.)

Morgan (*Chem. and Ind.*, 1938, **57**, 1191) found a wide variety of *N*-acylglucosamines to be capable of giving a positive Ehrlich test after alkali treatment. This was particularly important in the case of *N*-benzoyl- and *N*-trimethylacetyl-glucosamine, both of which are structurally capable of the above oxazole or oxazoline formation but not of pyrroline formation.

A close analogy is seen in the work of Wenker (*J. Amer. Chem. Soc.*, 1935, **57**, 1079; 1938, **60**, 2152) on the formation of oxazolines (IX) from *N*-acyl-β-amino-alcohols (VIII).



(VIII.) (IX.)

($R = CH_3$ or C_2H_5, R' = aliphatic hydrocarbon chain.)

Alternatively, Morgan (*loc. cit.*) suggested that two molecules of an *N*-acylglucosamine may condense to form a diglucopyrazine. This possibility must be admitted in view of the claim of Stolte (*B.Ph.P.*, 1907, **11**, 19) to have produced bistetrahydroxybutylpyrazine from glucosamine.

It seems clear, therefore, that glucosamine and its derivatives exhibit a pronounced tendency to give rise, by inter- and intra-molecular reaction, to heterocyclic derivatives of unusual type. In view of the possible existence of similar heterocyclic formations in proteins and of the importance of carbohydrates in the natural processes of protein synthesis, this behaviour of the amino-hexose merits further investigation.

(i) *The Action of Dilute Alkali Solution on* N-*Acetylglucosamine*.—The method of Zuckerkandl and Messiner-Klebermass (*loc. cit.*) for the preparation of *N*-acetylglucosamine has been modified and improved to give a 97% yield. The product, after heating with dilute alkali solution, gives a red-purple colour with Ehrlich's reagent. The present work was undertaken with a view to deciding whether this was due to the presence in the reaction mixture of a glucopyrroline, glucoxazole, glucoxazoline, or diglucopyrazine. The hygroscopic non-crystalline nature of those derivatives which may be presumed to possess the heterocyclic formulation and their great lability to acid and moderately concentrated alkali solution rendered impossible the use of many of the normal methods of carbohydrate chemistry. Much of the accumulated evidence is therefore of a circumstantial character, but it appears to confirm the assumption that on treatment with dilute alkali solution *N*-acetylglucosamine acquires a heterocyclic configuration, and requires that the heterocyclic ring so formed is of the oxazoline type.

Dr. Morgan has informed me of a method he has employed for the isolation of the chromophoric heterocyclic product present in the reaction mixture and the method has been utilised, with slight modifications, in the present work. At room temperature, dilute alkali solution produced no effect on *N*-acetylglucosamine, but at the boiling point the solution rapidly acquired the capacity of giving a positive Ehrlich test, the change being accompanied by some degradation of the sugar molecule. The degree of conversion of *N*-acetylglucosamine into the chromophoric substance was followed by the colorimetric method of Morgan and Elson (*loc. cit.*) and was found to be proportional to the amount of alkali present, up to one molecular equivalent. Two preparations, one containing an amount of 0·02N-potassium hydroxide constituting one equivalent of the *N*-acetylglucosamine present, the other containing one-fifth the amount of alkali, were treated identically. Colorimetric estimation indicated 100% final conversion in the first case and 20% in the second. Isolation of the product gave yields of 72% and 24% respectively.

The technique of preparing the chromophoric product followed similar lines for each alkali utilised, and the product was always an amorphous, hygroscopic powder, with a varied degree of coloration, and occasionally containing inorganic matter which could not be fractionated out. It gave a direct positive Ehrlich test and appeared to have a similar nature whatever the alkali used. In the main, when unchanged starting material, lower degradation products and inorganic matter had been as far as possible eliminated

by fractionation, the colour-producing material appeared to be essentially homogeneous. The distinction between this product and its parent *N*-acetylglucosamine rested on the fact that the former was hygroscopic, amorphous, very soluble in alcohols, had a low melting point (*ca.* 70°), a low specific rotation (*ca.* + 30°), exhibited no mutarotation, and gave a direct positive Ehrlich test. It was stable to boiling water, but in cold dilute acid or moderately concentrated alkali solution the capacity for giving a positive Ehrlich test was destroyed, presumably as a result of opening of the heterocyclic ring, since *N*-acetylglucosamine was recovered from such solutions. This ring rupture in concentrated alkali solution is probably responsible for the Fehling's reduction capacity of the chromophoric product.

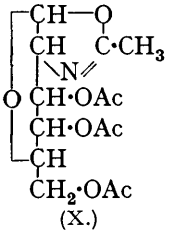
It has been indicated that the work of Morgan (*loc. cit.*) necessitated rejection of the glucopyrroline hypothesis of Zuckerkandl and Messiner-Klebermass (*loc. cit.*). Stereochemical considerations lead to the same conclusion and, in the present work, the result of molecular weight determinations and of chemical investigation suggested that neither the glucopyrroline nor the diglucopyrazine hypothesis had any basis in fact. Two alternative formulations then remained for the heterocyclic product, which, as already indicated, could be 2-*methyl*-4 : 5-*glucopyrano*-Δ^2-*oxazoline* (VII) or 2-*methyl*-4-$\alpha\beta\gamma\delta$-*tetrahydroxy*-n-*butyloxazole* (VI), the distinction between the two forms resting upon the presence or absence of the oxygen bridge in the hexosamine after alkali treatment.

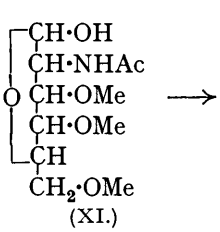
The oxazoline formulation implied the presence in the molecule of three free hydroxyl groups, an intact sugar bridge, and one double bond. The oxazole formulation required that the molecule should contain four free hydroxyl groups, no sugar bridge, and a pair of conjugated double bonds.

The product showed no mutarotation, indicating a fixed stereochemical position for the reducing group, and showed none of the spectrographic characteristics which would have resulted from the presence of conjugated double bonds or a pyrrole nucleus. Bromine oxidation gave glucosamine hydrobromide, indicating the hexosamine portion of the molecule to be intact. Hydrolysis of the chromophoric product with boiling methyl-alcoholic hydrogen chloride destroyed the colour-producing capacity and caused rapid reversion to *N*-acetylglucosamine, which was recovered in 80% yield. Methylation with methyl sulphate and alkali gave methyl 3 : 4 : 6-trimethyl *N*-acetylglucosamine—both results indicating that the *N*-acetyl group was still present in the molecule, linked to the reducing group in such a manner that its original nature could be easily restored.

The product was then acetylated with acetic anhydride in pyridine and the acetylated substance, which gave a positive Ehrlich test, was isolated as a hygroscopic glass which was not crystallised. Its *O*-acetyl content, estimated by the method of Kunz and Hudson (*J. Amer. Chem. Soc.*, 1926, **48**, 1982), indicated the presence of three acetyl groups and, with the other analyses, gave an empirical formula conforming to that of 2-*methyl*-4 : 5-(3′ : 4′ : 6′-*tri*-O-*acetylglucopyrano*)-Δ^2-*oxazoline* (X).

This result could not be explained if the original product were a glucoxazole or glucopyrroline. Further, molecular weight determinations by the ebullioscopic method on aqueous solutions of the chromophoric substance, and chloroform solutions of its triacetate, the apparatus of Swietoslawski (*Bull. Soc. chim.*, 1927, **41**, 717) being used, gave values favouring the unimolecular glucoxazoline structure and eliminating the diglucopyrazine hypothesis.


(X.)

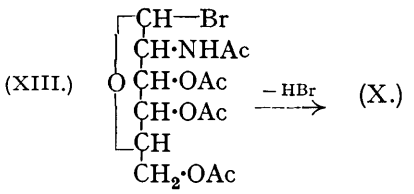

(XI.) (XII.)

N-Acetylglucosamine was then methylated by a technique based on that of Holden

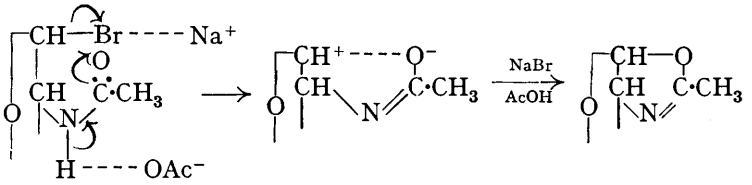
and West (*J. Amer. Chem. Soc.*, 1934, **56**, 930) for glucose. The resultant methyl 3 : 4 : 6-trimethyl *N*-acetylglucosaminide was converted into 3 : 4 : 6-trimethyl glucosamine hydrochloride, both of which products were prepared also by Cutler, Haworth, and Peat (J., 1937, 1979) by somewhat different methods. The hydrochloride was converted into 3 : 4 : 6-*trimethyl* N-*acetylglucosamine* (XI) with the reducing hydroxyl free to interact with the *N*-acetyl group on alkali treatment to form the heterocyclic 2-*methyl*-4 : 5-(3′ : 4′ : 6′-*trimethylglucopyrano*)-Δ^2-*oxazoline* (XII).

This product again gave a positive Ehrlich test, but despite thorough fractionation was obtained as a non-crystalline hygroscopic glass which decomposed on distillation. Methylation of the glucoxazoline by the method of Purdie and Irvine (J., 1903, **83**, 1028) gave a product with a methoxyl content only two-thirds that required by the above formulation. This partially methylated product was again a glass giving a positive Ehrlich test and, like the trimethyl glucoxazoline above, still capable of reducing Fehling's solution on heating. This again suggests linkage of the reducing group in the chromophoric substance in such a manner that it cannot be methylated under mild conditions.

Although this attempt to obtain the trimethyl glucoxazoline by two alternative routes failed, it was found possible to prepare the triacetyl glucoxazoline already described by a different method which avoided alkali degradation and permitted the isolation of a pure crystalline product. Penta-acetyl glucosamine was converted by the method of Moggridge and Neuberger (*loc. cit.*) into 1-bromo-3 : 4 : 6-tri-*O*-acetyl *N*-acetylglucosamine (XIII), which, on heating with one equivalent of sodium acetate solution, eliminated hydrogen bromide and was converted into the tri-*O*-acetyl glucoxazoline (X).



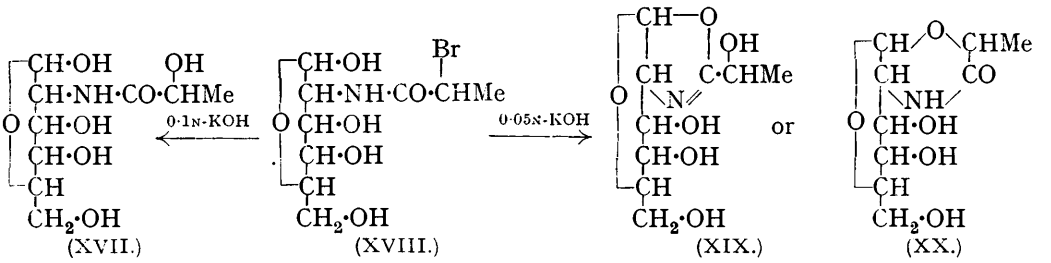
The solution remained colourless during the reaction, and the product was extracted from the aqueous solution with chloroform; it formed very minute, hygroscopic crystals on addition of dry ether to the concentrated extract. It gave a positive Ehrlich test and analyses and molecular weight determinations by Rast's method (*Ber.*, 1922, **55**, 1051) confirmed the above formulation. The nature of this reaction suggests that the mechanism of the heterocyclic ring formation follows the scheme depicted below (XIV; XV; XVI) where only the relevant portions of the molecule are shown :



A similar type of mechanism probably holds for the formation of the unsubstituted glucoxazoline by the action of alkali on *N*-acetylglucosamine.

(ii) *The Action of Dilute Alkali Solution on* N-α-*Bromopropionylglucosamine.*—*N*-α-Halogenoacylglucosamines again give a positive Ehrlich test after alkali treatment and *N*-α-bromopropionylglucosamine was found to yield a chromophoric product similar to that already discussed as resulting from alkali treatment of *N*-acetylglucosamine. This chromophoric product contained no halogen and although, in view of the work already described, it seemed improbable that it should be a glucopyrroline or diglucopyrazine,

it was still necessary to decide whether *N*-α-bromopropionylglucosamine gave rise to a glucoxazoline (XIX) or a glucoxazine (XX) with a six-membered heterocyclic ring.



(XVII.) (XVIII.) (XIX.) (XX.)

Molecular weight determinations confirmed the unimolecular nature of the product and acetylation gave a tri-*O*-acetyl derivative which could result only from a parent compound of structure (XX), *i.e.*, 3-*keto*-2-*methyl*-5 : 6-*glucopyrano*-3 : 4 : 5 : 6-*tetrahydro*-1 : 4-*oxazine*. Analyses and molecular weight determinations indicated the acetylated derivative to be 3-*keto*-2-*methyl*-5 : 6-(3′ : 4′ : 6′-*tri*-O-*acetylglucopyrano*)-3 : 4 : 5 : 6-*tetrahydro*-1 : 4-*oxazine*. Further, it was observed that, whereas *N*-α-bromopropionylglucosamine in 0·05N-alkali eliminated hydrogen bromide to form the glucoxazine (XX), the use of less dilute alkali replaced the halogen atom direct by hydroxyl to give N-α-*hydroxypropionylglucosamine* (XVII). This product was also obtained by treating the glucoxazine with concentrated alkali solution, the capacity for giving a positive Ehrlich test being simultaneously destroyed—a fact which is again in favour of the presence of a heterocyclic system labile to concentrated alkali solution.

It seems, therefore, a logical conclusion that in the presence of hot dilute alkali solution *N*-acyl- and *N*-α-halogenoacyl-glucosamines undergo intermolecular reaction in the manner originally suggested by Morgan and Elson (*loc. cit.*) with consequent formation of derivatives containing heterocyclic systems which are of the oxazoline and oxazine type respectively. In view of the importance of carbohydrates for the natural synthesis of proteins, the ease of formation of these products and their great reactivity and lability may well render the reaction one of some biological significance.

Experimental.

N-*Acetylglucosamine*.—50 G. of glucosamine hydrochloride, 37·5 g. of silver acetate, and 33·75 g. of acetic anhydride were shaken with 500 c.c. of absolute methyl alcohol for 3 hours at room temperature and then refluxed for 5 minutes, and the liquid filtered hot; the residue was washed twice with 250 c.c. of boiling water, the process being conducted in the absence of light up to this point. The combined filtrate and washings were acidified with 2 drops of concentrated hydrochloric acid, and the liquid filtered after standing at room temperature for 2 hours and concentrated to 50 c.c. at 45°/15 mm. 50 C.c. of alcohol and 100 c.c. of ether were added and the product was filtered off after 2 hours. Concentration of the mother-liquor and alcohol–ether precipitation gave a further yield. The product was dissolved in the minimum quantity of hot water, and alcohol added until its concentration was 90%, followed by sufficient ether to produce a faint turbidity; the white needles obtained (52 g.) had m. p. 196°, $[\alpha]_D^{18°}$ + 75° (init.) ⟶ + 41·2° (in water; const.) (Found : C, 43·5; H, 7·0; N, 6·1; $CO{\cdot}CH_3$, 19·25. Calc. for $C_8H_{15}O_6N$: C, 43·43; H, 6·8; N, 6·3; $CO{\cdot}CH_3$, 19·45%).

N-α-*Hydroxypropionylglucosamine*.—20 G. of *N*-α-bromopropionylglucosamine (Weizmann and Hopwood, *loc. cit.*) were heated at 100° with 1 l. of 0·1N-sodium hydroxide for 15 minutes, giving a dark brown solution, which was evaporated to dryness at 50°/15 mm. The residue was extracted with 250 c.c. of hot absolute methyl alcohol; the *product* crystallised in white needles when the extract cooled. The mother-liquor was evaporated to dryness at 40°/15 mm., and the residue again extracted as above, giving a further amount of product. The dark brown mother-liquor gave a positive Ehrlich test, but the presence of degradation products prevented the isolation of any glucoxazine.

The crystalline product was recrystallised from methyl alcohol, forming white needles (6·7 g.), m. p. 217°, $[\alpha]_D^{18°}$ + 69·1° (init.) ⟶ + 66·2° (const.; 0·068 g. in 5 c.c. of water) (Found : C, 43·5; H, 6·85; N, 5·5. $C_9H_{17}O_7N$ requires C, 43·0; H, 6·8; N, 5·75%). It was

soluble in hot water and hot alcohols but insoluble in ether and organic solvents. It reduced Fehling's solution on heating and, after heating with alkali, gave a positive Ehrlich test.

β-*Methyl* 3 : 4 : 6-*Trimethyl* N-*Acetylglucosaminide*.—32 G. of *N*-acetylglucosamine were methylated in 12, 10, and 10 g. portions, each dealt with as follows The sample was placed with 25 c.c. of water in a 2-litre flask immersed in a water-bath at 55°. Vigorous stirring was maintained throughout the reaction. 37·5 C.c. of methyl sulphate in 50 c.c. of carbon tetrachloride were added rapidly, followed by 162·5 c.c. of 60% sodium hydroxide solution at the rate of 1 drop per 2 seconds for 5 minutes, 1 drop per second for 5 minutes, then 3 drops per second until addition was completed. The bath temperature was raised to 75° and 56·5 c.c. of methyl sulphate were added (3 drops per second). After completion of this addition the bath temperature was raised to and maintained for 30 minutes at 100°. The cooled reaction mixture was refluxed with 300 c.c. of chloroform for 20 minutes, and the liquid filtered while hot, the chloroform extract being separated in the usual manner. The chloroform extracts from each methylation were combined. The aqueous filtrates and the sodium sulphate precipitates were then combined and refluxed for 20 minutes with a further 200 c.c. of chloroform; this extract was isolated as before and added to the previous extracts. The whole solution was then dried and evaporated to dryness at 40°/15 mm., and the residue recrystallised from ethyl acetate, forming white needles (34 g.), m. p. 192° (Found : C, 51·9; H, 8·4; N, 5·2; OMe, 44·7; $CO{\cdot}CH_3$, 15·5. Calc. for $C_{12}H_{23}O_6N$: C, 52·0; H, 8·3; N, 5·1; OMe, 44·8; $CO{\cdot}CH_3$, 15·5%). The product was non-reducing and gave no Ehrlich test.

3 : 4 : 6-*Trimethyl Glucosamine Hydrochloride*.—11·75 G. of methyl 3 : 4 : 6-trimethyl *N*-acetylglucosaminide were steam-hydrolysed for 2 hours with 400 c.c. of 4N-hydrochloric acid, the solution evaporated almost to dryness at 50°/15 mm., and the product isolated by adding 100 c.c. of methyl alcohol and 200 c.c. of ether. It was recrystallised from 75% alcohol and decomposed at 210°; yield, 8·4 g. (77%); $[\alpha]_D^{18°}$ + 54·8° (init.) ⟶ + 99·5° (const.; 0·237 g. in 10 c.c. of water) (Found : C, 42·0; H, 7·7; N, 5·5; OMe, 36·0; Cl, 14·15. Calc. for $C_9H_{20}O_5NCl$: C, 41·9; H, 7·8; N, 5·4; OMe, 36·1; Cl, 14·2%). It reduced Fehling's solution on heating, but gave no Ehrlich test until after condensation with acetylacetone. It was soluble in water and hot alcohols, but not in organic solvents.

3 : 4 : 6-*Trimethyl* N-*Acetylglucosamine*.—9·8 G. of 3 : 4 : 6-trimethyl glucosamine hydrochloride, 6·38 g. of silver acetate, 3·9 g. of acetic anhydride, and 200 c.c. of absolute methyl alcohol were shaken for 3 hours at room temperature, and the mixture then refluxed for 5 minutes, light being excluded until the completion of this stage. The liquid was filtered hot, and the residue washed twice with boiling alcohol. When the filtrate and washings cooled, the *product* crystallised in long white needles (6·0 g.), m. p. 234°, $[\alpha]_D^{18°}$ + 75° (init.) ⟶ + 44·8° (const.; 0·1473 g. in 10 c.c. of water) (Found : C, 49·9; H, 8·1; N, 5·7; OMe, 35·5; $CO{\cdot}CH_3$, 16·0. $C_{11}H_{21}O_6N$ requires C, 50·2; H, 8·0; N, 5·3; OMe, 35·4; $CO{\cdot}CH_3$, 16·1%). It was soluble in hot alcohols, in water, but not in ether or chloroform. It reduced Fehling's solution and gave a positive Ehrlich test after heating with dilute alkali solution.

2-*Methyl*-4 : 5-*glucopyrano*-Δ^2-*oxazoline*.—(a) *Colorimetric estimation*. The conversion of *N*-acetylglucosamine into the " glucoxazoline " was followed colorimetrically by comparison with standards prepared and utilised as follows : Five solutions were set up as standards, each consisting of 1 c.c. of water containing 0·2, 0·4, 0·6, 0·8, and 1 mg. of *N*-acetylglucosamine. Each solution was brought to p_H 11·3 by addition of 0·1 c.c. of buffer solution composed of 500 c.c. of 0·1N-sodium hydrogen phosphate and 7·5 c.c. of N-sodium hydroxide. These standards were heated on a boiling water-bath for 20 minutes and cooled, and 7·9 c.c. of glacial acetic acid and 1 c.c. of Ehrlich's reagent added. After 20 minutes the colour had developed to a stage suitable for comparison.

Estimations were conducted by removing such a quantity of reaction mixture as would, when diluted to 10 c.c., give a solution of concentration 1 mg. of *N*-acetylglucosamine plus glucoxazoline per c.c. To 1 c.c. of this solution were added 0·1 c.c. of buffer, 7·9 c.c. of glacial acetic acid, and 1 c.c. of Ehrlich's reagent. The colour intensity was compared with that of the standards after 20 minutes.

(b) *Preparation with an equivalent amount of* 0·02N-*potassium hydroxide*. 1 G. of *N*-acetylglucosamine, dissolved in 225 c.c. of 0·02N-potassium hydroxide, was heated on a water-bath at 75°. Conversion into the glucoxazoline—estimated as above—was 100% in 30 minutes. The resultant solution was evaporated to dryness at 40°/15 mm., the residue triturated with cold absolute methyl alcohol, and insoluble matter filtered off after remaining at 0° overnight. The precipitate was washed with ice-cold absolute methyl alcohol and the filtrate plus washings were evaporated to dryness in a vacuum over concentrated sulphuric acid. The residue

was triturated as above, insoluble matter (giving no Ehrlich test) filtered off after keeping at 0° overnight, and the filtrate and washings again evaporated to dryness as before. The residue was dissolved in the minimum quantity of cold absolute methyl alcohol, now going completely into solution. This solution gave no further precipitate on keeping for several days at 0° and an equal volume of acetone was added, followed by dry ether until no further product was precipitated. The flocculent precipitate was washed with dry ether by decantation, centrifuged, and dried in a vacuum over concentrated sulphuric acid. It had m. p. 70—75°, $[\alpha]_D^{18°}$ + 31·6° (0·038 g. in 5 c.c. of water). Yield, 0·66 g. (72%).

(c) *Preparation with one-fifth of the equivalent amount of* 0·004N-*potassium hydroxide.* 1 G. of *N*-acetylglucosamine, dissolved in 225 c.c. of 0·004N-potassium hydroxide, was heated on a water-bath at 75°, the extent of conversion into the glucoxazoline being 20% after 2 hours. This degree of conversion did not increase when the reaction mixture was maintained under these conditions for a further 5 hours; the chromophoric product was then isolated in the manner described above. Yield, 0·22 g. (24%). During the isolation, 0·58 g. of unchanged *N*-acetylglucosamine was recovered as distinct from the traces found in experiment (b).

(d) *Preparation with the equivalent amount of* 0·02N-*barium hydroxide.* 5·9 G. of *N*-acetylglucosamine in 1335 c.c. of 0·02N-barium hydroxide were heated at 90° for 30 minutes. The filtered solution was evaporated to dryness, and the glucoxazoline isolated as above, but despite its complete solubility in methyl alcohol the product contained 12·9% of ash. It was redissolved in the minimum quantity of absolute methyl alcohol, and barium precipitated as carbonate by treatment with carbon dioxide. The product was then isolated from the filtrate in the usual manner; yield, 2·63 g. (48·5%) (Found: C, 46·8; H, 6·8; N, 7·7; ash, 0·8; *M*, ebullioscopic in water, 190—200. $C_8H_{13}O_5N$ requires C, 47·2; H, 6·4; N, 6·9%; *M* 203).

(e) *Preparation with aqueous ammonia.* 7·25 G. of *N*-acetylglucosamine in 145 c.c. of water were treated with 1 c.c. of concentrated aqueous ammonia and kept at 75°. The conversion was 20% after 30 minutes and another 1 c.c. of aqueous ammonia was then added. After 1 hour the conversion was 40%. 5 C.c. of aqueous ammonia were added and the conversion was 100% after 3 hours. The product was isolated as described; $[\alpha]_D^{18°}$ + 30·9° (0·168 g. in 10 c.c. of methyl alcohol). Yield, 4·2 g. (58%).

In each of the above preparations the product was an amorphous, extremely hygroscopic powder, varying in colour from light to dark brown. It was readily soluble in water and cold alcohols but insoluble in ether, acetone, and organic solvents. It gave a direct positive Ehrlich test, which capacity was destroyed by solution in cold weak acid or moderately concentrated alkali solution but not by boiling in water for 2 hours. It reduced Fehling's solution and exhibited no mutarotation. It gave no osazone or phenylhydrazone and gave no additive complexes with bromine, methyl iodide or acyl halides. Oxidation with permanganate completely destroyed the molecule and acetylation or benzoylation with the acid chloride in pyridine at room temperature resulted in such extensive decomposition that no recognisable products were isolated. Even after thorough fractionation, the glucoxazoline contained some inorganic matter and an appreciable amount of degraded sugar products responsible for the coloration. In consequence, the analytical values could not be regarded as conclusive.

(f) *Bromine oxidation.* 1 G. of bromine was added to 0·2088 g. of glucoxazoline in 5 c.c. of water, and the mixture kept at room temperature for 3 days; the bromine was then distilled off at room temperature and the solvent at 40°/15 mm. The residue was recrystallised from hot ethyl alcohol, forming white needles, acid to litmus, reducing Fehling's solution on heating, and giving a negative Ehrlich test. After condensation with acetylacetone the product gave a positive Ehrlich test. $[\alpha]_D^{18°}$ + 59° (const.; water). Tiemann (*Ber.*, 1886, **19**, 156) records + 59·6° for glucosamine hydrobromide (Found: C, 27·5; H, 5·5; N, 5·6; Br, 30·5. Calc. for $C_6H_{13}O_5N,HBr$: C, 27·7; H, 5·4; N, 5·4; Br, 30·7%).

(g) *Hydrolysis with* 0·02N-*methyl-alcoholic hydrogen chloride.* 0·93 G. of glucoxazoline was refluxed for 3 hours with 250 c.c. of 0·02N-methyl-alcoholic hydrogen chloride until the solution no longer gave a positive Ehrlich test. The reaction mixture was then neutralised with barium carbonate, and the filtrate evaporated to dryness at 40°/15 mm., giving a brown residue (0·81 g.; 80%). The product was dissolved in hot methyl alcohol and decolorised with norit, the filtrate evaporated to dryness, and the residue recrystallised from methyl alcohol. The product was identified as *N*-acetylglucosamine.

(h) *Methylation with methyl sulphate and alkali.* 1·8 G. of glucoxazoline in 5 c.c. of water were placed in a flask immersed in a water-bath at 55°, and rapid stirring commenced. 5·2 C.c. of methyl sulphate in 10 c.c. of carbon tetrachloride were added rapidly, followed by 25 c.c. of 60% sodium hydroxide solution at the rate 1 drop per 2 seconds for 5 minutes, 1 drop per

second for 5 minutes, then 3 drops per second until the addition was complete. The bath temperature was raised to 75°, 9·2 c.c. of methyl sulphate added (3 drops per second), and the temperature maintained at 100° for 30 minutes after completion of this addition. The cooled solution was extracted four times with 10 c.c. portions of chloroform, the combined extracts dried and decolorised with norit, and the filtrate concentrated to small volume under reduced pressure. On addition of light petroleum the product crystallised; it was recrystallised from the same solvents, forming feathery needles (0·5 g.), m. p. 192°. It was identified as methyl 3 : 4 : 6-trimethyl *N*-acetylglucosaminide.

(i) *Methylation with methyl iodide and silver oxide.* 10 C.c. of methyl iodide were refluxed for 6 hours with 3·8 g. of glucoxazoline dissolved in the minimum amount of dry methyl alcohol, with hourly additions of half-gram portions of silver oxide. The liquid was then filtered, the residues washed with methyl alcohol, and the filtrate plus washings evaporated to dryness at 40°/15 mm. The residual syrup was remethylated and reisolated in the same manner, and the process repeated until ten methylations had been given. The 4th—6th methylations were carried out in methyl alcohol–acetone, the 7th and 8th in acetone, and the 9th and 10th in methyl iodide alone. The methoxyl content remained constant at 22·6% after the 7th methylation (Calc. for a trimethyl glucoxazoline, 37·9%). The product was a brown syrup which did not crystallise, gave a positive Ehrlich test, reduced Fehling's solution on heating, and decomposed on distillation at low pressure.

2-*Methyl*-4 : 5-(3′ : 4′ : 6′-*tri*-O-*acetylglucopyrano*)-Δ^2-*oxazoline*.—(a) *By direct acetylation.* 1·09 G. of glucoxazoline were shaken for 3 hours with 25 c.c. of dry pyridine and 25 c.c. of acetic anhydride, then kept at room temperature for 24 hours The reagents were distilled off at 40°/15 mm. to give a light brown syrup, which was reacetylated and reisolated in a similar manner and dried to a glass in a vacuum over concentrated sulphuric acid. The product was hygroscopic, gave a positive Ehrlich test, and was soluble in chloroform, acetone, ethyl acetate and alcohol, but insoluble in ether and light petroleum. It reduced Fehling's solution on heating. Fractionation from a variety of organic solvents failed to yield a crystalline product and low-pressure distillation resulted in decomposition. Yield, 0·95 g. (63%); $[\alpha]_D^{18°}$ + 36·7° (0·038 g. in 5 c.c. of chloroform) (Found : C, 49·6; H, 5·9; N, 4·2; *O*-Ac, 39·0; *M*, ebullioscopic in chloroform, 320. $C_{14}H_{19}O_8N$ requires C, 51·1; H, 5·8; N, 4·25; *O*-Ac, 39·2%; *M*, 329).

(b) *From* 1-*bromo*-3 : 4 : 6-*tri*-O-*acetyl*-N-*acetylglucosamine.* 3 G. of 1-bromo-3 : 4 : 6-tri-*O*-acetyl-*N*-acetylglucosamine were heated with 1 g. of sodium acetate in 50 c.c. of water at 65°, the conversion into the heterocyclic product being followed by the change of rotation, initial value + 115°, constant final value + 23° after 6 hours. The solution, which then gave a positive Ehrlich test, was extracted six times with 25 c.c. portions of chloroform and the combined extracts were dried, concentrated to 15 c.c. at 30°/15 mm., treated with dry ether, and kept at 0° overnight. The product crystallised in minute white needles, which were washed with dry ether by decantation, centrifuged, and dried in a vacuum over concentrated sulphuric acid. It was slightly hygroscopic, reduced Fehling's solution, and gave a positive Ehrlich test. Yield 1·3 g. (54%); m. p. 70°, $[\alpha]_D^{18°}$ + 54° (0·21 g. in 10 c.c. of chloroform) (Found : C, 51·0; H, 5·65; N, 4·2; *O*-Ac, 39·1; *M*, Rast, 325. $C_{14}H_{19}O_8N$ requires C, 51·1; H, 5·8; N, 4·25; *O*-Ac, 39·2%; *M*, 329).

2-*Methyl*-4 : 5-(3′ : 4′ : 6′-*trimethylglucopyrano*)-Δ^2-*oxazoline*.—2·3 G. of 3 : 4 : 6-trimethyl *N*-acetylglucosamine were heated on a boiling water-bath with 437·3 c.c. of 0·02N-barium hydroxide for 30 minutes; the solution was then concentrated to 100 c.c. at 45°/15 mm. and treated with carbon dioxide, and the filtrate evaporated to dryness. The residue was dissolved in 20 c.c. of absolute methyl alcohol, 40 c.c. of acetone added, and the solution filtered after 1 hour. The filtrate and acetone washings of the precipitate were evaporated to a syrup, and the process repeated. The reisolated syrup was triturated with acetone, and the filtrate evaporated to a syrup, which was dissolved in chloroform. Ethyl acetate was then added, and the filtrate again evaporated to a syrup; this was triturated with ethyl acetate, and the filtrate treated with a little dry ether. The final filtrate was evaporated to a syrup, from which no further matter could be removed which did not give a positive Ehrlich test. This syrup gave a positive Ehrlich test, reduced Fehling's solution on heating, and decomposed on distillation. It was soluble in chloroform, acetone, ethyl acetate, alcohol and water and sparingly soluble in ether and light petroleum. Yield, 1·1 g. (51·5%) (Found : C, 53·5; H, 7·1; N, 5·5; OMe, 35·1. $C_{11}H_{19}O_5N$ requires C, 53·9; H, 7·75; N, 5·7; OMe, 37·9%).

3-*Keto*-2-*methyl*-5 : 6-*glucopyranotetrahydro*-1 : 4-*oxazine*.—(a) *Preparation with* 0·05N-*potassium hydroxide.* 4 G. of *N*-α-bromopropionylglucosamine were heated on a boiling water-bath

with 250 c.c. of 0·05N-potassium hydroxide for 15 minutes; conversion into the glucoxazine was then 100% as estimated colorimetrically. The solution was evaporated to dryness at 40°/15 mm., the residue extracted with cold absolute methyl alcohol, and the filtrate evaporated to dryness in a vacuum over concentrated sulphuric acid. Insoluble matter was removed by the fractionation process described for the glucoxazoline and the chromophoric product was precipitated and isolated in a similar manner as a hygroscopic, amorphous, pale yellow powder (1·9 g.), m. p. 140—145°.

(b) *Preparation with 0·05N-barium hydroxide.* 16·75 G. of *N*-α-bromopropionylglucosamine were heated as above for 30 minutes with 1 l. of 0·05N-barium hydroxide, the solution evaporated to dryness, and the chromophoric product isolated as above. Yield, 12·4 g. (theoretical); m. p. 140—145°, $[\alpha]_D^{18°}$ + 19·4° (0·1254 g. in 5 c.c. of water). Mol. wt. (ebullioscopic in water), 238 (calc., 233).

In each case the product was an amorphous, hygroscopic powder which reduced Fehling's solution on heating and gave a positive Ehrlich test, which property was destroyed by cold acid or moderately concentrated alkali solution. Its general properties were similar to those of glucoxazoline. Spectrographic analysis in water indicated the absence of any characteristic absorption phenomena. It was soluble in water and alcohols but not in organic solvents.

(c) *Conversion into N-α-hydroxypropionylglucosamine.* 1·18 G. of glucoxazine were dissolved in 45 c.c. of 13% sodium hydroxide solution at room temperature. Crystals appeared after 5 minutes and after 1 hour the capacity of the solution for giving a positive Ehrlich test had been destroyed. The solution was kept at 0° overnight, and the crystals then filtered off, washed with ice-cold water and recrystallised from methyl alcohol. The product was identified as *N*-α-hydroxypropionylglucosamine. Yield, 0·6 g. (49%).

(d) *Hydrolysis with 1% methyl-alcoholic hydrogen chloride.* 0·53 G. of glucoxazine was refluxed for 3 hours with 20 c.c. of 1% methyl-alcoholic hydrogen chloride, the capacity for giving a positive Ehrlich test being gradually destroyed. The crystals which appeared were filtered off, a further yield being obtained on addition of dry ether to the mother-liquor. The combined product was recrystallised from dilute alcohol, and the product identified as glucosamine hydrochloride. Yield, 0·2 g. (40%).

(e) *Methylation with methyl iodide and silver oxide.* 3·9 G. of glucoxazine were given twelve methylations with methyl iodide and silver oxide in the manner described for the glucoxazoline, the methoxyl content remaining constant at 28·5% after the methylation (calc. for a trimethyl glucoxazine, 33·8%). The product was a light brown syrup which gave a positive Ehrlich test, reduced Fehling's solution on heating, and decomposed on distillation.

3-Keto-2-methyl-5 : 6-(3′ : 4′ : 6′-tri-O-acetylglucopyrano)tetrahydro-1 : 4-oxazine.—3·46 G. of glucoxazine were acetylated with 37·5 c.c. of dry pyridine and 37·5 c.c. of acetic anhydride as described for the glucoxazoline, and the product (a syrup) isolated similarly. It was triturated with chloroform, the filtrate treated with 2 vols. of ethyl acetate, and insoluble matter filtered off after 1 hour. The filtrate was evaporated to dryness under reduced pressure, the resultant syrup triturated with ethyl acetate, the filtrate evaporated to dryness, and the residue dissolved in chloroform. The addition of light petroleum then precipitated the acetylated glucoxazine as a light brown, hygroscopic powder, which gave a positive Ehrlich test and reduced Fehling's solution on heating. It was soluble in water, alcohols, ethyl acetate, chloroform and acetone but not in ether or light petroleum. Yield, 1·9 g. (36%); $[\alpha]_D^{18°}$ + 32·1° (0·081 g. in 5 c.c. of chloroform) (Found : C, 49·7; H, 5·8; N, 3·9; *O*-Ac, 36·6; *M*, Rast, 362. $C_{15}H_{21}O_9N$ requires C, 50·1; H, 5·85; N, 3·9; *O*-Ac, 35·9%; *M*, 359).

The author wishes to express his gratitude to Professor T. Campbell James, D.Sc., for his invaluable supervision of this work; to Dr. W. T. J. Morgan, who permitted initiation of the work at the Lister Institute, Elstree; and to Professor W. N. Haworth, F.R.S., for accommodation at the University of Birmingham Chemistry Department throughout the Session 1938—1939. The author wishes also to express his indebtedness to the University College of Wales, Aberystwyth, for the Sir Garrod Thomas Fellowship; to the Department of Scientific and Industrial Research for a maintenance grant; and to the University of Wales for a University Fellowship.

THE EDWARD DAVIES CHEMICAL LABORATORIES,
THE UNIVERSITY COLLEGE OF WALES, ABERYSTWYTH. [*Received, January 25th,* 1940.]