39. *Conversion of* 2-p-*Toluenesulphonyl β-Methylglucoside into Methyl* epi*Glucosamine.*

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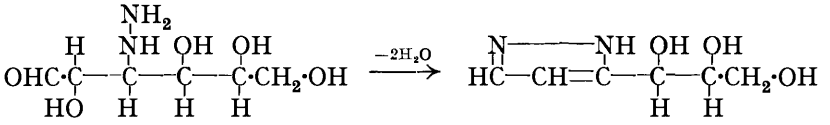
A sugar base, designated methyl *epi*glucosamine, first prepared by Fischer, Bergmann, and Schotte (*Ber.*, 1920, **53**, 509) by treatment of methylglucoside 2-chlorohydrin with hot concentrated aqueous ammonia, is identical with the amino-compound isolated during the present investigation. Fischer, discussing its probable mode of formation, suggested that elimination of the halogen may result in the formation of an ethylene oxide ring, which then opens to add on ammonia. He reported great difficulty in removing the glucosidic methoxyl group even with hot concentrated hydrochloric acid. Levene and Meyer (*J. Biol. Chem.*, 1923, **55**, 221), however, found that hydrolysis of the methoxyl group proceeds readily with even dilute acid, but the resultant amino-sugar quickly loses water to form a non-reducing anhydro-amino-compound. The free sugar was shown to exist in dilute aqueous acid solution, from which an osazone was prepared. The osazone contained five atoms of nitrogen and had thus been formed without elimination of the amino-group. This fact proved that the basic group was not attached to the second carbon atom and Levene suggested position 3 as an alternative.

The fact that the amino-group had not replaced the halogen directly supports the suggestion of Fischer that an anhydro-compound may form an intermediate stage in the preparation, as the breaking of the anhydro-ring would permit the entering amino-group to become attached to either of the two carbon atoms involved. This also makes it possible that the configuration of the groups on both carbon atoms involved may change during the reaction. Levene expressed no opinion as to the probable configuration of either of the two and thus left four possibilities open. The compound could be a 3-amino-derivative of glucose, mannose, allose, or altrose.

Later Freudenberg, Burkhart, and Braun (*Ber.*, 1926, **59**, 714) prepared an amino-glucose by a method which left no room for doubt as to the position of the basic residue. The *p*-toluenesulphonyl derivative of diacetone glucose was treated with ammonia to yield a diacetone amino-compound, which was hydrolysed to 3-amino-glucose. The osazone and the methylglucoside prepared from this base were both different from the corresponding derivatives of *epi*glucosamine and the source of difference between the two compounds was sought.

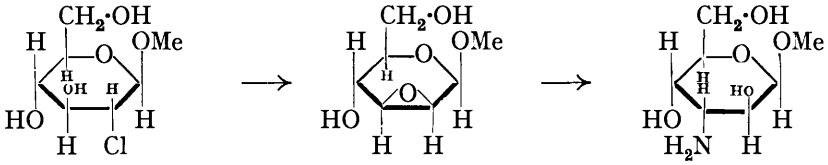
Formulation of *epi*glucosamine as a 3-amino-hexose was justified by the preparation

of a hydrazine derivative by treatment of the methylglucoside 2-chlorohydrin of Fischer with hydrazine. The halogen was replaced by a hydrazine residue in a manner analogous to the preparation of methyl *epi*glucosamine, but the product was not isolated. Removal of the methoxyl group from this substance by acid hydrolysis resulted in its condensation to give glycerylpyrazole :



The formation of this compound reveals the position taken up by the hydrazine and it is reasonable to assume that the amino-group is introduced in a similar manner.

The difference between *epi*glucosamine and the new amino-compound of Freudenberg, Burkhart, and Braun is ascribed by them to a difference of configuration, and the probable configuration of the former is deduced from its method of formation as follows : In the methylglucoside 2-chlorohydrin the configuration of the third carbon atom is known, and the formation of the intermediate ethylene oxide compound does not involve any change in the bond between this carbon and its oxygen. Consequently the intermediate anhydro-compound must have the mannose configuration. Rupture of this ring with addition of one molecule of ammonia probably involves inversion at carbon atom 3, where the nitrogen enters, but not at 2. If this reasoning be sound, it leads to the formulation of methyl *epi*glucosamine as 3-aminomethylaltroside.



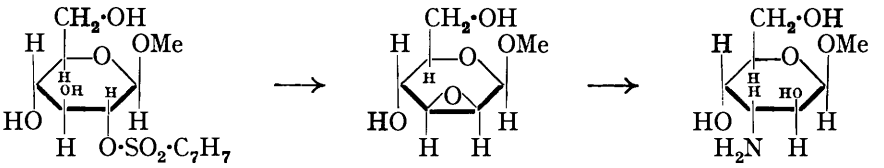
The present investigation was undertaken with the intention of effecting a synthesis of glucosamine by direct substitution of an amino-group into a suitable glucose derivative.

A glucose derivative was desired having the hydroxyl in position 2 substituted by an acidic radical which could be replaced by an amino-group. 3 : 4 : 6-Triacetyl β-methylglucoside (Brigl, *Z. physiol. Chem.*, 1921, **116**, 1 ; 1922, **122**, 245) was therefore converted into 2-*p*-toluenesulphonyl 3 : 4 : 6-triacetyl β-methylglucoside. This compound could be deacetylated with either sodium methoxide or methyl-alcoholic ammonia, giving 2-*p*-toluenesulphonyl β-methylglucoside (Reynolds, J., 1931, 2627; 1933, 224). Both these compounds were crystalline and, on treatment with hot methyl-alcoholic ammonia, both yielded a syrup from which could be isolated the crystalline hydrochloride of an amino-methylhexoside.

This hydrochloride bore a close resemblance to the methyl *epi*glucosamine hydrochloride of Fischer. Hydrolysis with either concentrated or dilute hydrochloric acid resulted in very little rotation change and no development of reducing power, although evaporation of the solution indicated that a reaction had occurred to give an anhydro-amino-compound similar to that described by Levene and Meyer (*loc. cit.*). An osazone prepared under the conditions specified by these authors was shown by analysis to be the phenylosazone of an amino-hexose hydrochloride. The identity of these products with methyl *epi*glucosamine and anhydro*epi*glucosamine respectively is beyond question. It is thus evident that the replacement of the *p*-toluenesulphonyl by an amino-group has involved a migration, probably *via* the formation of an anhydro-compound, as was observed by Ohle and Vargha (*Ber.*, 1929, **62**, 2435) in their study of the elimination of the *p*-toluenesulphonyl group from 6-*p*-toluenesulphonyl monoacetone glucose.

Sodium methoxide did not remove the *p*-toluenesulphonyl group from 2-*p*-toluenesulphonyl 3 : 4 : 6-triacetyl β-methylglucoside under the conditions employed in the deacetylation. The formation of the amino-glucoside from this material almost certainly

involves the intermediate stage of an anhydro-compound, which is possibly, in view of the following evidence, the 2 : 3-anhydro-β-methylmannoside. The reaction may therefore be represented as follows :



The anhydro-amino-hexose obtained by hydrolysis of methyl *epi*glucosamine was assumed by Levene and Meyer (*loc. cit.*), without evidence, to be a 1 : 2-anhydro-3-amino-compound. The only well-authenticated 1 : 2-anhydro-sugar derivative is the 3 : 4 : 6-triacetyl 1 : 2-anhydroglucose of Brigl (*loc. cit.*), which is characterised by instability in presence of water. In striking contrast, the present anhydro-amino-compound is formed in aqueous solution and is stable.

Removal of the amino-group from methyl *epi*glucosamine hydrochloride by means of silver nitrite or nitrous acid proceeded smoothly, as indicated by the rotation change. The product obtained from the action of silver nitrite was non-reducing and, on acetylation, gave a syrup which was probably a tetra-acetyl methylhexoside. Methylation of the de-aminated product gave a syrup which was mainly a tetramethyl methylhexoside. Hydrolysis of the unsubstituted de-aminated material resulted in a rotation change to an equilibrium value which was in close agreement with the recorded rotation of altrose. The methylaltroside, tetra-acetyl methylaltroside, and tetramethyl methylaltroside are unknown.

If Freudenberg's deduction for the possible configuration of methyl *epi*glucosamine be assumed and the compound is actually 3-aminomethylaltroside, removal of the amino-group with nitrous acid will yield β-methylaltroside or β-methylmannoside according as the reaction does not or does involve inversion at carbon atom 3. If the configuration of the second carbon atom in methyl *epi*glucosamine is not as in altrose and the amino-compound is actually 3-aminomethylglucoside or 3-aminomethylalloside, de-amination may yield a methylalloside or a glucoside. No constants are recorded for allose, methylalloside, or tetra-acetyl or tetramethyl methylalloside.

From the above evidence it is possible, therefore, that the action of nitrite on methyl *epi*glucosamine hydrochloride produced β-methylaltroside, but, as the identification of this material depends solely upon the rotation of its hydrolysis product, this conclusion must be regarded as tentative.

Experimental.

2-p-*Toluenesulphonyl* 3 : 4 : 6-*Triacetyl* β-*Methylglucoside.*—*p*-Toluenesulphonyl chloride (12 g.) was added to a solution of 3 : 4 : 6-triacetyl β-methylglucoside (10 g., prepared by Brigl's method, *loc. cit.*, p. 245) in dry pyridine (30 c.c.). After 12 hours at 20°, the liquid and crystals were mixed with a large volume of water. The product, washed until free from pyridine and recrystallised from alcohol, formed fine colourless needles, m. p. 157°, $[\alpha]_D^{20°}$ + 1·5° in chloroform (*c*, 2·0); yield, 50% (compare Reynolds, J., 1931, 2627) (Found : C, 50·9; H, 5·7. Calc. for $C_{20}H_{26}O_{11}S$: C, 50·6; H, 5·5%).

2-*p*-Toluenesulphonyl β-methylglucoside was prepared from this substance by the method of Reynolds (J., 1933, 225).

Methyl epi*Glucosamine Hydrochloride.*—This substance can be obtained equally well from 2-*p*-toluenesulphonyl β-methylglucoside or from its triacetate, but, as the latter is more accessible, only the reaction with it need be described. The triacetate (2·0 g.) was dissolved in methyl alcohol (40 c.c.) saturated with ammonia at 0°, and heated in a sealed tube at 110° for 18 hours. After cooling, the clear yellow solution was evaporated under diminished pressure to a syrup, which was heated at 100°/15 mm. for 2 hours. Much acetamide was thereby removed. The dark brown syrup was next dissolved in alcohol (20 c.c.), and addition of ether to the cooled solution precipitated crystalline ammonium *p*-toluenesulphonate (identity confirmed by analysis). The filtered solution was evaporated to a syrup, which was again dissolved

in alcohol (10 c.c.). Dry hydrogen chloride, passed into the cooled solution, at once precipitated a crystalline product, which was washed with a little cold alcohol and recrystallised from methyl alcohol, giving colourless needles of methyl *epi*glucosamine hydrochloride (Fischer, *loc. cit.*). This product was readily soluble in water, the solution containing ionised chlorine; m. p. 210—212° (decomp.), $[\alpha]_D^{20°}$ — 145° in water (*c*, 0·9) (yield, 40%) (Found : C, 36·6; H, 7·4; N, 6·4; OMe, 13·0. Calc. for $C_7H_{16}O_5NCl$: C, 36·6; H, 7·0; N, 6·1; OMe, 13·5%).

The reaction between methyl-alcoholic ammonia and 2-*p*-toluenesulphonyl 3 : 4 : 6-triacetyl β-methylglucoside at 15° proceeds in 72 hours only so far as the formation of 2-*p*-toluenesulphonyl β-methylglucoside and may be utilised for the preparation of this substance (yield, 75%).

Hydrolysis of methyl *epi*glucosamine hydrochloride with boiling hydrochloric acid of any concentration greater than 1% led to the formation of anhydro*epi*glucosamine hydrochloride, which was isolated by evaporation of the acid solution under diminished pressure (yield, 80%); m. p. 228° (decomp.) after darkening at 200°, $[\alpha]_{5780}^{20°}$ — 184° in water (*c*, 0·7) (Found : C, 36·6; H, 6·4; N, 6·9. Calc. for $C_6H_{12}O_4NCl$: C, 36·6; H, 6·1; N, 7·1%).

Hydrolysis of methyl *epi*glucosamine hydrochloride to *epi*glucosamine was effected by 8 hours' boiling with 0·5% hydrochloric acid. After cooling, sodium acetate, phenylhydrazine, and acetic acid were added and the mixture was heated at 100° for 3 hours. On cooling, the hydrochloride of *epi*glucosamine phenylosazone separated as a yellow crystalline mass; recrystallised from alcohol, it had m. p. 225—227° (decomp.) (Found : C, 54·7; H, 6·4; N, 17·5. Calc. for $C_{18}H_{23}O_3N_5$,HCl : C, 54·9; H, 6·1; N, 17·7%).

Deamination of Methyl epi*Glucosamine Hydrochloride.*—The hydrochloride (0·5 g.) in water (10 c.c.) was shaken with freshly recrystallised silver nitrite (0·6 g.) for 15 hours, and the silver chloride then removed and washed with a little water. The solution and washings were combined and diluted to 50 c.c. The rotation became constant 24 hours after the addition of the silver nitrite. The specific rotation at this stage was approximately $[\alpha]_D$ — 52°, calculated on the basis of complete conversion of the methyl *epi*glucosamine into a methylhexoside. The dissolved silver was next removed by addition of a very slight excess of hydrochloric acid. The filtrate was immediately neutralised with aqueous sodium hydroxide and evaporated under diminished pressure. A little inorganic impurity was removed by dissolving the syrup in alcohol. The product (0·25 g.) did not reduce Fehling's solution until after hydrolysis with boiling hydrochloric acid. The hydrolysis product had $[\alpha]_D$ — 100° (approx.) in water.

The above deamination product (mainly methylhexoside) gave a syrupy tetra-acetyl derivative when heated with acetic anhydride in the presence of anhydrous sodium acetate (yield, 40%), $[\alpha]_D$ — 22° in chloroform (Found : OMe, 8·8%). The product obtained by the deamination of 2·5 g. of the aminomethylhexoside was methylated by methyl sulphate and sodium hydroxide in the usual way. After completion of the methylation by methyl iodide and silver oxide the product (1·2 g.) was distilled, giving a colourless mobile syrup (0·4 g.), b. p. 105°/0·2 mm., $n_D^{18°}$ 1·4586, $[\alpha]_D^{20°}$ — 47° in chloroform (*c*, 1·8), — 32° in water (*c*, 1·7). This consisted mainly of a tetramethyl methylhexoside, but it did not appear to be identical with any known fully methylated hexose (Found : C, 52·2; H, 9·1; OMe, 56·6. $C_{11}H_{22}O_6$ requires C, 52·8; H, 8·8; OMe, 62·0%). The small yield of methylated product indicated that the deamination process had been accompanied by side reactions.

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