

INVESTIGATION ON THE ADDITION COMPOUNDS
OF THE CARBOHYDRATES.

by

WILLIAM J. HEDDLE, B.Sc.

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**INVESTIGATION ON THE ADDITION COMPOUNDS
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I N T R O D U C T I O N.

The interaction between sugars and alkalis has been known for a great number of years. As early as 1807, Ramsay (1) obtained crystals of a compound of sucrose and lime and thirty years later Peligot (2) prepared and analysed a number of compounds of sucrose and the "earths". In spite of the fact that such compounds have been known and studied for over a century, their constitution has not yet been satisfactorily determined.

The majority of workers in this field, have employed physicochemical measurements. Madsen (3), Hirsch and Schlags (4), Hirsch (5), Stearn (6) and Urban and co-workers (7) have all sought to prove by various physical measurements that sugars in the presence of alkali act as weak acids. For further details of this work the reader is referred to the Ph.D. Thesis of G.C. Ritchie (8).

Although these conductivity experiments do show the feeble acid nature of the sugars and the probability of chemical combination between the organic and inorganic constituents in aqueous solution, yet it is not possible to isolate these complexes from that medium as is the case with alkaline earth sugar compounds. The latter are

definite crystalline solids which are insoluble in water and stable towards it, whilst those of the alkali metals are, in general, white, amorphous, deliquescent powders which are rapidly attacked by moisture and thus cannot be prepared from aqueous solution.

Groot (9,10.) showed that such compounds appeared to be formed even in aqueous solution and he observed that the maximum depression in the specific rotation in glucose solutions took place when mono-molecular proportions of the sugar and alkali were present. Groot expressed the view that these changes were due to the formation of an unstable compound. $C_6H_{11}O_6K$ followed by Lobry de Bruyn - Van Eckenstein transformations and it was further suggested that the effects of alkali on glucose may be attributed to the enolisation of the glucose molecule.

The cause of the apparent acidity and the point at which it arises in the molecule cannot be satisfactorily determined by such methods, although Michaelis and Rona (11) on the basis of potentiometric measurements of hydrogen ion concentrations of solutions of alkali hydroxides and sugars, suggested

3.

the possibility that the acidity was due to the presence of enolic forms $-\text{CH}(\text{OH}) = \text{C}(\text{OH})-$. This is only applicable to the reducing sugars however and does not explain the formation of addition compounds with sucrose.

As can be seen from the above physicochemical determinations there existed no very definite ideas as to the structure of these sugar alkali compounds and the results simply serve to show that the sugars examined were capable of removing alkali or hydroxyl ions from the solution.

There appears to be three main possibilities as to the chemical nature of the products we are considering.

Firstly, they may not be chemical compounds at all. By mixing aqueous solutions of sucrose and lime (13) precipitates of widely varying compositions in which no definite relationship between the constituents is apparent, may be readily obtained on the addition of alcohol. But, on the other hand, that over eighty authenticated compounds have been isolated from the various carbohydrates and alkalis in which the constituents appear to be present in stoicheio-

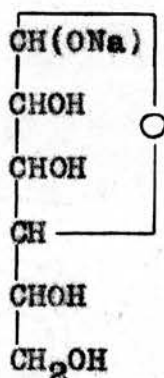
metric proportions seems to indicate a case of definite chemical combination rather than mere adsorption or fortuitous precipitation of the metallic constituent along with the carbohydrate. Further, it is found that when both sugar and hydroxide are soluble in the reaction medium simple mixing of the solutions is sufficient to precipitate the compound. This would seem to be clearly a case of chemical reaction.

Secondly, these sugar alkali products are considered by some workers, especially those in the field of polysaccharides, to be adsorption compounds. In the case of alkali cellulose, for example, the inconsistencies found in the analyses of the products obtained by various authors were so great that adsorption phenomena seemed the only explanation.

Thirdly, the carbohydrate and alkali may combine to form a definite chemical compound which may be either (a) of an alcoholate nature or (b) an addition complex.

(a). If an example of the first alternative then such substitution products should be comparable with those of the alcohols, e.g. sodium ethoxide. König

and Rosenfeld (14) claimed to have isolated the compound $C_6H_{11}O_6Na$ which they obtained from glucose and sodium ethoxide in alcoholic solution. Zemplén and Kunz (15) were of the opinion that if such a compound did exist it should have the structure



and as such should prove capable of great synthetic use, especially in the syntheses of the glucosides and disaccharides. But all efforts to effect such syntheses have failed and the existence of an alcoholate structure was therefore considered doubtful. (b). If, of the second type, then the alkali residue will have become attached to some reactive point within the sugar molecule and the weight of evidence seems to be in favour of this alternative. A considerable impetus was given to this theory when Zemplén and Kunz

were able to show that actually an addition compound of the type $C_6H_{12}O_6, NaOC_2H_5$ was formed by glucose and sodium ethoxide, the ethoxide residue being established by a qualitative test.

Percival (17) confirmed the existence of this compound and isolated the corresponding sodium methoxide addition complex. He showed, too, that if a trace of water were present, the compounds isolated contained no combined alcoholic residue owing to the hydrolysis of the sodium ethoxide, which may explain the results of the earlier workers. From potassium hydroxide and glucose in absolute alcoholic solution Percival obtained an addition compound to which he gave the formula $C_6H_{12}O_6, KOH$ derived by analogy from the sodium alkoxide compounds and which was supported by analytical data. Further work in this field by Percival (18) and Percival and Ritchie (19) has resulted not only in the isolation of a number of such addition compounds of the sugars but also in the determination of their structure.

Thus it would appear that the compounds formed between the sugars and the alkali hydroxides are of the addition compound type and somewhat analogous to the

to the known crystalline sugar metallic salt compounds such as glucose-sodium chloride (20), glucose-potassium chloride (21), sucrose-sodium chloride (22), and the two isomeric mannose-calcium chloride compounds (23).

It is necessary to decide the positions within the sugar molecule of the reactive points at which the inorganic constituents become attached before investigating the type of linkage involved. It is generally assumed that, in the case of a reducing sugar, the reducing group is the most active and would be concerned in any union with alkali. This, however, is not based on any direct evidence.

From the fact that Marchlewski (12) was unable to obtain glucose phenylosazone from phenylhydrazine and sodium glucosate it does not necessarily follow that the alkali metal was attached to the reducing group, since in the normal production of glucosazone from glucose and phenylhydrazine, acidification with acetic acid is necessary. Further, the isolation of addition compounds of non-reducing sugars such as sucrose and the methylglucosides does show definitely that the reducing group is not necessarily the one involved.

It was shown by Percival, however, that by the selective action of a reagent such as dimethyl sulphate on the sugar alkali compounds, it was possible to obtain partially substituted sugars from which, by the isolation of definite reference compounds, the original position of the alkali group could be decided. By this method he showed that, in the case of the product formed between glucose and potassium hydroxide, the alkali constituent was actually intimately connected with the reducing group. Further work by Percival (18) and by Percival and Ritchie (19) has shown that in all the cases examined the alkali is closely connected with one or more hydroxyl groupings of the sugar molecule. The evidence being based on the isolation of crystalline acetates and osazones.

The purpose of this work is to extend this method of examination to the potassium hydroxide compounds of other carbohydrates, the monosaccharides, α - and β -methylglucosides, the disaccharide, maltose and the polysaccharides, amylose and cellulose.

PART I.

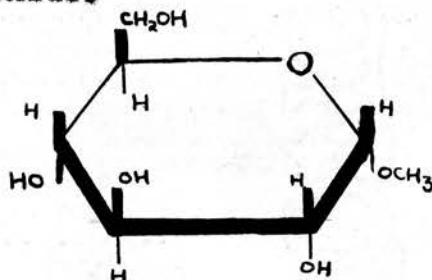
**THE ADDITION COMPOUNDS OF α -METHYLGLUCOSIDE
AND POTASSIUM HYDROXIDE.**

B I B L I O G R A P H Y.

- (1). Ramsay Nicholson's Journal, 18.9 (1807).
- (2). Peligot Compt. Rendu., 1838, 6, 232.
- (3). Madsen Z. physik chem., 1901, 36, 290.
- (4). Hirsch and Schlags.. Z. physik chem., 1929, A141,
387.
- (5). Hirsch Z. ang. chem., 1929, 68, 160.
- (6). Stearn J. physic. chem., 1931, 35,
2226.
- (7). (Urban and Williams.. J. Biol. Chem., 1933, 100, 237.
(Urban and Schaffer. J. Biol. chem., 1932, 94, 697.
- (8). Ritchie Thesis Edin. Univ., 1936.
- (9). Groot Biochem Z., 1924, 146, 72.
- (10). Groot Biochem Z., 1927, 180, 341.
- (11). Michaelis and Rona.. Biochem Z., 1913, 47, 232.
- (12). Marchlewski Ber., 1893, 26, 2928.
- (13). MacKenzie and Quin..J.C.S., 1929, 951.
- (14). Hönig and Rosenfeld..Ber., 1877, 10, 871.
- (15). Zemplén and Kunz ...Ber., 1923, 56, 1705.
- (17). Percival J.C.S., 1934, 1160.
- (18). Percival J.C.S., 1935, 648.
- (19). Percival and
Ritchie J.C.S., 1936, 1765.
- (20). Beilstein 4 Aufl. Bd. 1. S. 893.
- (21). V. Lippmann "Chemie der Zuckerarten",
1904, 548.
- (22). Gill J.C.S., 1871, 24, 269.
- (23). Dale J.A.C.S., 1929, 51, 2788.

DISCUSSION.

α -methylglucoside, owing to its non-reducing properties, was chosen for the first investigation since it had been shown that both sucrose (1) and α -methylgalactoside (2) were capable of combining with potassium hydroxide.



α -methylglucoside

In the cases of the addition compounds of glucose (3), cellobiose, lactose and galactose (4) it was shown that the reducing group of the sugar molecule was always an active participant in union with alkali irrespective of whether other hydroxyls were or were not concerned. Galactose, for instance, was found to combine with one molecular proportion of potassium hydroxide, that being associated with the reducing group. Yet α -methylgalactoside, in which the reducing group is blocked, was found to be capable of combination with potassium hydroxide too. Glucose, in a similar manner, was shown to form a mono alkali compound in which the

Reducing group was again concerned and it was decided to investigate the alkali combining capacity of the analogous β -methylglucoside. Sucrose forms many compounds with metallic oxides and hydroxides, some of which are of industrial importance, but the great reactivity of sucrose in this direction does not extend to other sugars in which the reducing group is blocked, although raffinose shows similar tendencies (5).

There are no references recorded in the literature showing the isolation of compounds of the methylglucosides with the alkalis and indeed, the non-existence of such compounds is reported by some workers. Percival (3) could find no evidence for the formation of addition compounds of α - and β -methylglucosides with potassium hydroxide and MacKenzie and Quin (6) were not able to observe any compound formation between the methyl- glucosides, - fructosides and -maltosides and calcium hydroxide. Ritchie (2), however, was able to isolate an alkali addition compound of α -methylgalactoside and showed that one molecular proportion of potassium hydroxide was associated with one molecule of sugar and that the alkali residue was closely connected with the hydroxyl grouping attached

to the second carbon atom in the α -methylgalactoside molecule. In lactose (4), i.e. 4-galactoside glucose it was shown that the non reducing portion of the disaccharide was apparently more able to combine with alkali than the reducing portion. This result suggests that galactose, which was shown to be capable of forming a mono-alkali associated addition compound only, in glycosidic union is able to attract more potassium hydroxide residues than in the case of galactose itself, although much may depend on other influences such as solubility and stability in the equilibrium solution.

It was found that α -methylglucoside in alcoholic solution gave no precipitate with alcoholic potassium hydroxide but on the addition of dry ether a copious white precipitate was produced and this is evidently the reason why compound formation had not been observed previously (3,6.). On examination this was shown to be the alkali addition compound of α -methylglucoside and potassium hydroxide. Ether had not been used before in the attempts to isolate the addition compounds of α - and β -methylglucosides.

Unfortunately it was not possible to employ satis-

factorily the titration method as applied by Percival (3) for estimating indirectly the alkali combining capacity of the sugar, owing to the solubility of the addition complex in the equilibrium solution even on the addition of a considerable volume of dry ether and it was found necessary to make direct analyses of the dried isolated products obtained using various concentrations of alcoholic potash. These results are tabulated in TABLE I (p 15). Theoretically for a compound of the type $C_7H_{14}O_6.KOH$, 100 gm. α -methylglucoside would require to combine with 28.9 gm. potassium hydroxide or ^{the} potash content in the compound is 22.4%. It will be seen from a preliminary examination of the table that such a compound was apparently formed and although no great reliance can be placed in the accuracy of these figures it was considered sufficient evidence to indicate that α -methylglucoside combined with potassium hydroxide, the extent of combination being such that one molecule of alkali combined with one molecule of the glucoside.

The method used for determining the structure of this addition compound (and the other addition compounds discussed in this thesis) depends on the intro-

duction of a stable group in place of the more loosely held alkali. This was accomplished by controlled methylation of the alkali addition compound under mild conditions and was effected by means of dry dimethyl sulphate, previously neutralised with anhydrous potassium carbonate, in the absence of a solvent (3). By a single treatment with this reagent for as short a time as possible it is found that for each alkali residue initially combined with the sugar a corresponding number of methyl groups are introduced into the sugar molecule. The partially methylated sugar is then examined to assign the position of the methyl groups thus indicating the structure of the original alkali addition compound.

There is the possibility however, that, during the treatment with methyl sulphate, the alkali residue may migrate to a new position and thus cause the introduction of the methyl groups in positions different from that originally concerned. In order to minimise this care is taken to have both reacting substances as dry as possible. But even in the presence of water or other ionising solvents the method does not necessarily fail, since it is to be expected that the alkali will have

TABLE - I

α -methylglucoside-potassium hydroxide.

Conc. of α -methylglucoside %	1.00	0.95	7.50	5.60	5.00	3.80	3.00	2.50	2.10	1.67	1.36	1.15
Initial Normality of KOH in the original glucoside-alkali solution before addition of ether.	1.43	1.21	0.900	0.550	0.600	0.450	0.360	0.300	0.269	0.200	0.165	0.138
%age potassium hydroxide in isolated product.	16.4	20.3	24.0	21.3	22.7	20.4	18.8	19.2	17.6	17.6	16.7	14.9

Calculated for $C_7H_{14}O_6 \cdot KOH$, KOH, 22.4%

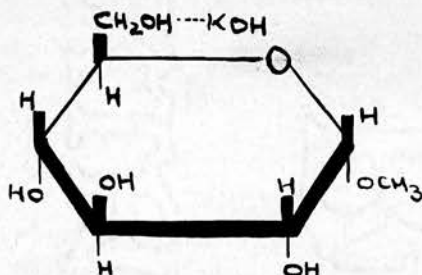
or, 100 gm. α -methylglucoside would combine with 28.9 gm. potassium hydroxide.

associated with the hydroxyl groups of maximal acidity and such would be unalterable during such mild treatment.

The dry alkali addition compound was methylated in the manner indicated and by treatment of the product in methyl alcoholic solution with alcoholic potash and ether, the unchanged α -methylglucoside was removed from the sphere of reaction by precipitation. The residual solution was then taken to dryness and acetylated. In this manner a triacetyl monomethyl α -methylglucoside was obtained in 7% yield calculated on the weight of α -methylglucoside initially present. Attempts at crystallisation were not successful. On deacetylation and subsequent hydrolysis to remove the glucosidic methoxyl a methylated glucose was obtained and this was found to give a phenylosazone which proved to be identical with the phenylosazone of 6-methyl glucose. As was seen in the cases of cellobiose (4) and sucrose (1) it was to be expected that substitution would occur in the primary alcoholic residue owing to that grouping being probably the most vulnerable in the molecule.

It is apparent then that α -methylglucoside is capable of combining with one molecule of potassium hydroxide to form a compound in which the alkali residue is closely associated with the primary alcoholic grouping on the terminal carbon atom of the α -methylglucoside

molecule. The position of the potassium hydroxide residue in the addition compound is indicated in the formula



Thus, in spite of the reducing group, always an active participant in the union with alkali, being blocked, the glucose residue was still capable of uniting with alkali. This diacidic nature of glucose is in entire agreement with the view of Hirsch and Schlage (11) who, in a series of conductometric analyses were able to show the weak acidic nature of the sugars and drew up a table giving the dissociation constants of the important sugars. Those quoted for glucose at 25°C, were $k_1 = 7.8 \times 10^{-13}$ and $k_2 = 1.54 \times 10^{-14}$.

It is to be noted that the mild treatment of the alkali addition compound with dimethyl sulphate is not a case of methylation comparable with the usual standard method of methylating a sugar when the latter is treated with alkali and methyl sulphate. If such were

the case then by methylating a mechanical mixture of the sugar concerned with the proportionate quantity of potassium hydroxide under the same conditions of time and temperature, products identical to those produced above should be obtained. In a trial experiment Percival (1) subjected finely powdered potassium hydroxide and glucose in monomolecular proportions to methylation with dry neutral dimethyl sulphate under the same conditions as employed when treating the addition compounds. A careful examination of the products of the reaction revealed that the highest yield of methylglucoside was never greater than 0.5% of the weight of glucose initially present as compared with yields of about 20% when the addition compound of glucose was used (3). It was also pointed out by Ritchie (2) that under the experimental temperature conditions employed dimethyl sulphate in the absence of alkali had no action whatever on cellobiose, the sugar being recovered unchanged from the reaction mixture. It was shown also that methylation of the alkali addition compound with methyl iodide instead of methyl sulphate gave a poor yield of inferior product. It thus appeared that methyl iodide was not so efficient a reagent for this work.

The yield of monomethyl methylglucoside (7%) is seen to be much lower than the yield of methylglucoside obtained on methylating potassium hydroxide-glucose (3) (20%). It is, therefore, legitimate to conclude that the potassium hydroxide complex is much less stable when the primary alcoholic residue is concerned than when the reducing group is involved as in the case of glucose.

THE PREPARATION OF α -METHYLGLUCOSIDE.

α -methylglucoside was prepared by the method of Patterson and Robertson (10). Pure anhydrous glucose (500 gm.) was refluxed with anhydrous methyl alcohol (1030 gm.) containing dry hydrochloric acid gas (30gm) for 15 hours. On cooling crystals of crude α -methylglucoside separated out which were then recrystallised from methyl alcohol. Fine white needles were obtained.

Yield (crude) 320 grams.

The mother liquor was concentrated resulting in several crops of crystals each becoming progressively more contaminated with the β -isomer, the last crop containing about 33% of β -methylglucoside.

An attempt was made later to obtain some β -methylglucoside from the last crop but proved unsuccessful owing to the similarity in behaviour of the two isomers and was finally abandoned in favour of another method of preparation (12).

A melting point determination was made m.p. 164°C.

A rotation in water gave $[\alpha]_D^{15^\circ} + 155^\circ$. (c = 0.50).

EXPERIMENTAL.

THE ALKALI COMBINING CAPACITY OF α -METHYLGLUCOSIDE.

It was found that the "indirect" method as employed by Percival (3) for estimating the alkali combining capacity of a sugar could not be applied satisfactorily in this case. A few trial experiments were made but the results were inconclusive and recourse had to be made to direct analysis of the dried isolated addition complexes.

To an aqueous solution (1 c.c.) of a weighed quantity of recrystallised α -methylglucoside (1.5 gm.) a measured volume of absolute alcohol (20 c.c.) was added, followed after shaking by a known quantity of standard alcoholic potassium hydroxide solution (10.0c.c.) and dry ether (300 c.c.) till no further precipitation occurred. After standing for 15 minutes the precipitated alkali-sugar complex was filtered off through a Gooch crucible, washed with the standardised minimum quantity of absolute alcohol (1 c.c.) and dry ether (1 c.c.) to remove adhering potash solution and dried in a vacuum desiccator over phosphorus pentoxide. The alkali content of the addition complex was then determined by titration of a weighed quantity (.573 gm.)

against $N/10$ acid (23.20 c.c.) and phenolphthalein indicator.

$$\% \text{-KOH} = \frac{56.1 \times 100 \times 0.10 \times 23.20}{1000 \times 0.573} = 22.7\% \text{KOH.}$$

The results of a series of similar experiments are tabulated in Table I (p.15).

A TYPICAL PREPARATION OF α -METHYLGUCOSIDE POTASSIUM HYDROXIDE.

α -methylglucoside (20 gm.) was dissolved in water (10 c.c.) and warm absolute alcohol added (250 c.c.) almost to precipitation point. To the cold mixture a solution of potassium hydroxide (11 gm.) in absolute alcohol (100 c.c.) was added, the mixture shaken and then dry ether (700 c.c.) till no further precipitation occurred, well shaken again and then corked and set aside for 15 minutes. The white precipitated product was filtered quickly and washed as rapidly as possible with absolute alcohol (25 c.c.) to remove any adhering potassium hydroxide solution, followed by washing with dry ether (25 c.c.). The addition product was then transferred to a porous plate and dried over phosphorus

pentoxide in a vacuum desiccator.

Yield 22 grams.

The product was a white, amorphous, very deliquescent powder, rapidly becoming a brown sticky mass on exposure to the air.

Analysis:-

By titration against $N/10$ H_2SO_4 the alkali content was found to be 21.3% potassium hydroxide.

Calculated for mono alkali addition

compound $C_7H_{14}O_6$, KOH

KOH 22.4%

A TYPICAL METHYLATION OF α -METHYLGUCOSIDE POTASSIUM HYDROXIDE.

To the finely powdered α -methylglucoside potassium hydroxide compound (22 gm.) in a flask fitted with a stirrer, dimethyl sulphate (100 c.c.), which had been previously neutralised with anhydrous potassium carbonate, was added. The contents of the flask, surrounded by a water bath maintained at $60^\circ C$. for 5 minutes and then at $75^\circ C$. for 15 minutes were vigorously stirred.

Towards the end of the second period of heating the powdery solid had coagulated to a sticky and viscous mass. After cooling in ice the supernatant liquid was decanted off and the residual mass washed free from dimethyl sulphate with acetone, then extracted with boiling anhydrous methyl alcohol (50 c.c.), after which the cold extract was allowed to stand for three hours to allow the potassium methyl sulphate to separate out. After removal of this by filtration a solution of potassium hydroxide (6 gm.) in absolute alcohol (40 c.c.) was added, followed by dry ether till no further precipitation occurred. Addition of more alkali showed that all the unchanged α -methylglucoside had been removed. The precipitate (16 gm.) was filtered off, dried and stored in a vacuum desiccator for future methylations. The ethereal filtrate was acidified with glacial acetic acid and, after the removal of the precipitated potassium acetate, was taken to dryness under reduced pressure on a water bath maintained at 40°C.

ACETYLATION OF THE METHYLATED SYRUP.

The dried mass of syrup and potassium acetate was heated with a mixture of acetic anhy^dride (50 c.c.) and anhydrous sodium acetate (9.0 gm.) for 2½ hours at 95°C.

By pouring the cold product into cold water (400 c.c.) and neutralising with sodium bicarbonate then thoroughly extracting with chloroform (300 c.c.) and drying over anhydrous sodium sulphate and evaporating under reduced pressure at 45°C. a light brown syrup with non-reducing properties was obtained. Attempts at crystallisation were not successful.

Yield 1.09 grams.

Purification was effected by distillation in a high vacuum. A clear syrup (0.74 gm.) distilling at 185-195°C./0.05 mm. bath temperature was obtained.

Analysis:-

Found: OMe, 17.5% CH₃CO, 36.5%.

Calc. for a triacetylmethyl methylglucoside C₁₄H₂₂O₉.

OMe, 18.6%. CH₃CO, 38.6%.

$\left[\alpha \right]_{D}^{16^{\circ}} = +112^{\circ} \text{C.} \quad (c = 0.5) \text{ in chloroform.}$

DEACETYLATION AND HYDROLYSIS OF SYRUP.

The acetylated syrup (0.7 gm.) was deacetylated by Zemplén's method (13). To an ice-cold chloroform solution (10c.c.) of the syrup (1.0 gm.) an anhydrous methyl alcoholic solution (7 c.c.), of sodium (0.2 gm.) was added and the

mixture set aside in a beaker of cold water for three hours. At the end of this period a slight excess of acetic acid was added and the deacetylated product extracted with water (15 c.c.), followed by treatment of the chloroform extract with more water (15 c.c.) and of the aqueous extract with more chloroform (5 c.c.) The aqueous extracts were combined and carefully evaporated under diminished pressure on a water bath at 50°C. till the volume of the solution was reduced to suitable bulk. A sample of this was shown to be ~~non-reducing~~ towards Fehling's solution.

Sufficient dilute hydrochloric acid was added to bring the concentration of the acid to 5% and then heated on a water bath maintained at 95°C. till constant rotation was attained. The period required was six hours. The free mineral acid was neutralised with silver carbonate and the solution filtered. The silver residues were then thoroughly extracted with hot water and the extracts added to the original filtrate and the whole carefully evaporated under reduced pressure to a suitable volume.

FORMATION OF THE OSAZONE.

To the solution (10 c.c.) of the hydrolysed syrup pure phenylhydrazine (1.0 c.c.), crystalline sodium

acetate (0.5 gm.) and glacial acetic acid were added and the mixture heated on a water bath at 90°C. A small quantity of sodium bisulphite was added as recommended by Hamilton (14) to minimise the formation to tarry oxidation products. Within 2½ hours a precipitate had formed which was filtered off, washed thoroughly with dilute acetic acid and water and dried in vacuo. The filtrate on heating for a further period yielded a second crop of osazone. The crude product was purified by recrystallisation from aqueous alcohol.

Yield 0.08 grams.

m.p. 178-182°C.

This osazone was identified by means of mixed melting points with authentic specimens of 3-methyl glucosazone and 6-methyl glucosazone.

melting point of 3-methyl glucosazone	175°C.
mixed m.p. with 3-methyl glucosazone	158-162°C.
melting point of 6-methyl glucosazone	180-182°C.
mixed m.p. with 6-methyl glucosazone	180°C.
mixed m.p. of 6-methyl and 3-methyl glucosazone	162-3°C.

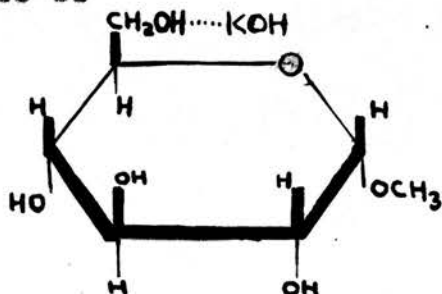
- The melting point of 6-methyl glucosazone is quoted as 177°C. by Helferich and Becker (15) and 178-179°C. by

Kuhn and Ziese (16) and recently Bell (7) has isolated a specimen melting at 190°C. 3-methyl glucosazone is quoted by both Freudenberg and Hixon (8) and Anderson, Charlton and Hawerth (9) to have a melting point of 178°C.

From the fact that the m.p. of the monomethyl glucoaszone was only slightly depressed on admixture with an authentic specimen of 6-methyl glucosazone, the two were considered identical.

S U M M A R Y.

1. It was shown that the alkali addition compound of α -methyl glucoside was thrown out of solution only on addition of ether to the alcoholic solution of α -methylglucoside containing potassium hydroxide.
2. Direct analysis of the dried isolated alkali addition complexes obtained by using various concentrations of alkali indicated the formation of a compound in which one molecule of potassium hydroxide was associated with one molecule of α -methylglucoside.
3. Controlled methylation, followed by acetylation resulted in the isolation of a triacetylmethyl methylglucoside in 7% yield.
4. Subsequent treatment of the acetate yielded a monomethyl glucosazone which was identified as 6-methyl glucosazone.
5. From a consideration of all the facts the structure of the potassium hydroxide addition compound of α -methylglucoside was considered in all probability to be



B I B L I O G R A P H Y.

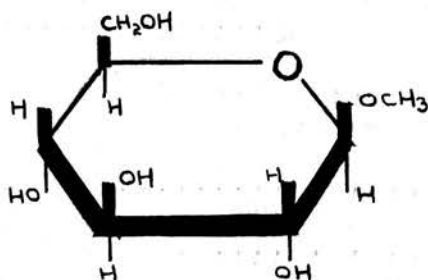
- (1) Percival J.C.S., 1935, 648.
- (2) Ritchie Thesis - 1936. (Edin.)
- (3) Percival J.C.S., 1934, 1160.
- (4) Percival and Ritchie J.C.S., 1936, 1765.
- (5) Beythien and Tollens Ann., 1889, 225, 199.
- (6) MacKenzie and Quin J.C.S., 1929, 951.
- (7) Bell J.C.S., 1936, 859.
- (8) Freudenberg and Hixon Ber., 1923, 56, 2126.
- (9) Anderson, Charlton and
Haworth J.C.S., 1929, 1329.
- (10) Patterson and Robertson J.C.S., 1929, 300.
- (11) Hirsch and Schlags Z. Physikal chem.,
1929, A, 141, 387.
- (12) Heddle This Thesis, P. 33.
- (13) Zemplen and Kunz Ber., 1923, 56, 1705.
- (14) Hamilton J.A.C.S., 1934, 56,
487.
- (15) Helferich and Becker Ann., 1924, 440, 13.
- (16) Kuhn and Ziese Ber., 1926, 59, 2314.

PART II.

**THE ADDITION COMPOUNDS OF β -METHYLGLUCOSIDE
AND POTASSIUM HYDROXIDE.**

DISCUSSION.

Attention was next directed towards the corresponding β -methylglucoside. The reducing group, which has been shown by Percival (1,2) and Percival and Ritchie (3) to be always an active participant in union with alkali, is again blocked.



β -methylglucoside.

Little information on the interaction of alkali and β -methylglucoside is recorded in the literature and no worker appears to have isolated any such complexes. Indeed the non-existence of such compounds is reported by some workers. MacKenzie and Quin (4) stated that over a series of observations no compound formation took place between β -methylglucoside and calcium hydroxide whilst Percival (1) was unable to find any evidence for the formation of addition compounds of β -methylglucoside with potassium hydroxide. Since it had been shown that α -methylglucoside was capable of combination with potassium hydroxide (5) it was decided to investigate the case of β -methylglucoside.

It was found that β -methylglucoside, like α -methylglucoside gave no precipitate with alcoholic potassium hydroxide solution. On the addition of ether, however, a white powder was precipitated which appeared to be an addition compound of β -methylglucoside and potassium hydroxide.

β -methylglucoside was prepared from α -glucose pentacetate (6) by the action of hydrobromic acid in glacial acetic acid solution upon the latter and then by treatment of the α -acetobromoglucose so formed with silver carbonate in an methyl alcoholic medium, tetraacetyl β -methylglucoside was obtained in good yield.

The glucoside was readily obtained by deacetylation. It was found more convenient, however, to deacetylate and form the additive complex simultaneously by using a suitable amount of potassium hydroxide.

Owing to the solubility in alcohol of the addition compound the alkali combining capacity of the sugar could not be determined by the method usually employed. (1) and one of direct analysis of the dried isolated compounds had to be used. By the treatment of β -methylglucoside with potash over a few trial concentrations it was evident that compound formation did occur (see Table I) and a series of experiments was

made over a range of alkali concentrations 1.93 N. to 0.46 N. potassium hydroxide, employing the acetate, to determine the alkali combining capacity and most suitable conditions for the isolation of the addition product. The results obtained are tabulated in Table II. (p35). Although no great accuracy for these figures can be claimed they seem to indicate that a compound of the type $C_7H_{14}O_6.KOH$ is formed. Theoretically the potassium hydroxide content for such a monoalkali addition compound is 22.4%.

The alkali addition product was subjected to the action of dry neutral dimethyl sulphate in the usual manner (1) and after solution of the reaction powder in hot methyl alcohol the unchanged β -methylglucoside was eliminated as far as possible by precipitation with alcoholic potassium hydroxide. Acetylation of the filtrate and distillation in a high vacuum gave rise to a triacetylmonomethyl β -methylglucoside with $[\alpha]_{\lambda}^{180} -19.4^\circ$ in chloroform. (Yield 4%). It was noticed that during methylation rapid stirring and thorough mixing of the reactants was essential otherwise hydrolysis took place and the acetylated product was found to have a positive rotation.

TABLE - I. β -methylglucoside potassium hydroxide.

conc. of β - methylglucoside - %	8.50	5.59	5.26	4.19
Initial Normality of KOH in the orig- inal glucoside- alkali solution before addition of ether.	1.51	1.10	0.631	0.248
%-age potassium hydroxide in isolated product.	18.7	18.6	20.7	21.4%

calculated for $C_7H_{14}O_6.KOH$, KOH, 22.4%.

or, 100 gm. β -methylglucoside would combine with
28.9 gm. potassium hydroxide.

TABLE - II

 β -methylglucoside-potassium hydroxide

conc. of tetra- acetyl β -methyl glucoside - %.	8.60	4.44	2.80	3.78	3.08	1.57	4.41
						2.50	

Initial normality of KOH in the orig- inal glucoside- alkali solution before addition of ether.	1.43	0.887	0.857	0.755	0.615	0.572	0.463
						0.500	

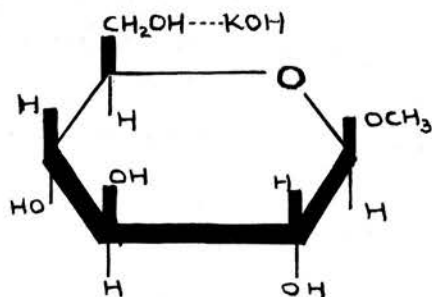
%-age potassium hydroxide in isolated product	18.8	22.2	21.9	22.1	21.4	22.2	22.5
						21.8	

calculated for $C_7H_{14}O_6 \cdot KOH$, KOH, 22.4%.

or, 100 gm. β -methylglucoside would combine with 28.9 gm. potassium hydroxide.

By deacetylation and hydrolysis, followed by treatment of the product with phenylhydrazine a mono-methyl osazone was obtained which was found to melt at 177-181°C. after several recrystallisations and it was identified as 6-methylglucosazone by means of mixed melting points with an authentic specimen of that phenylosazone.

From these results it was concluded that one molecule of β -methylglucoside is capable of combining with one molecule of potassium hydroxide to form an addition-compound in which the alkali residue is closely connected with the primary alcoholic grouping on the terminal carbon atom of the β -methylglucoside molecule. The structure of the alkali addition compound β -methylglucoside is then



The results of these experiments are in complete harmony with those obtained with α -methylglucoside and show that a glucose in which the reducing group is

blocked is still capable of union with alkali, the hydroxyl of maximal acidity being that of the terminal primary alcoholic grouping. It may be pointed out too that substitution occurred in the corresponding β -glucopyranose unit in the same position in the cellobiose molecule (3) and a knowledge of these structures might possibly be of importance with the constitution of alkali cellulose. As in the case of α -methylglucoside-potassium hydroxide the amount of conversion to the 6-methyl ether is low (4%) indicating the instability of the complex.

EXPERIMENTAL

THE PREPARATION OF TETRA-ACETYL
 β -METHYLGLUCOSIDE.

The tetra-acetate of β -methylglucoside was prepared from glucose by the method of Koenigs and Knorr (6) slightly modified. There are three stages.

(a) Conversion of glucose to α -glucose pentacetate.

Anhydrous glucose (200 gm.) was added to a mixture of anhydrous sodium acetate (100 gm.) and acetic anhydride (930 c.c.) and the whole heated on a water bath maintained at a temperature of 50°C. till nearly all the solid had dissolved. This was assisted by occasional agitation. The temperature was then slowly raised to 100°C. and maintained there for 2 hours. A copious white precipitate was obtained when the cold solution was poured into a large volume of ice-cold water (5 litres) containing finely powdered ice and neutralised with sodium bicarbonate. The crude acetate was filtered off, washed with cold water and dried in a desiccator over phosphorus pentoxide. Recrystallisation was effected from alcohol when fine white needle shaped crystals were obtained.

Yield (recryst.) 200 grams.

m.p. 108-109°C.

$[\alpha]_{\lambda = D}^{16^{\circ}C.}$ 97.4° in chloroform (c=0.68)

(b) Conversion of α -glucose pentacetate to α -aceto-bromoglucose.

A glacial acetic acid solution (32 c.c.) recently saturated at zero with dry bromine free hydrogen bromide gas was added to a glacial acetic acid solution (32 c.c.) of very finely divided dry glucose pentacetate (27 gm.) and shaken mechanically till all the solid had dissolved. After standing in the cold for two hours, the acetobromoglucose was extracted with cold chloroform (125 c.c.), then by pouring the chloroform layer into ice cold water containing powdered ice it was found that by vigorous stirring the yellow chloroform solution became colourless. The chloroform extract was then washed with water and after neutralisation with sodium bicarbonate, with more water and finally dried over anhydrous sodium sulphate. The solvent was carefully evaporated off at 45°C. under reduced pressure when a hard white solid was obtained which was left in contact with low boiling petrol ether (40/60°C.) overnight. The solid was removed and dried in vacuo.

Yield	18 grams.
m.p.	70-75°C.

(c). Conversion of α -acetobromoglucose to tetracetyl β -methylglucoside.

The α -acetobromoglucose (18 gm.) was dissolved in dry methyl alcohol (300 c.c.) and shaken mechanically for 12 hours with well-dried finely divided silver carbonate (45 gm.) until no bromine ions could be detected in the solution. The mixture was then filtered and after the silver residues had been thoroughly washed with water and the washings added to the original filtrate, the combined aqueous extract was concentrated to a thin syrup under reduced pressure at 45°C. This crystallised on standing. The colourless needles were washed free of adhering syrup with a little alcohol.

Yield 10 grams.

Recrystallisation was effected from methyl alcohol.

m.p. 104°C.

$[\alpha]_D^{12}$

-18.3° in chloroform
(c = 0.86).

THE ALKALI COMBINING CAPACITY OF
 β - METHYLGLUCOSIDE.

A. FIRST METHOD.

The alkali combining capacity of the free glucoside was determined. The method employed for deacetylation was Zemlén's (7).

β -methylglucoside tetra-acetate (1.0 gm.) was dissolved in chloroform (10 c.c.) and when ice-cold was added to a similarly cooled solution of sodium (0.25 gm.) in dry methyl alcohol (10 c.c.) and the whole immersed in a beaker of cold water. After standing for three hours the solution was neutralised with acetic acid and the deacetylated sugar extracted with cold water (10 c.c.). The chloroform layer was treated with a further quantity of water (5 c.c.) and the aqueous layer added to the first. The combined aqueous extracts were then shaken up with more chloroform (5 c.c.) and separated. The solution of the deacetylated sugar was taken to dryness and purified from the inorganic material by crystallisation from methyl alcohol.

Yield 0.35 grams.

m.p.

$[\alpha]_{D}^{180C}.$ -29.8° in water (c=0.50)

The alkali addition compound was obtained by solution of the β -methylglucoside (0.30 gm.) in absolute alcohol (3.7 c.c.) to which was added alcoholic potash (2.0 c.c. of 1.80 N. solution) followed by dry ether till no further precipitation occurred. After standing for 15 minutes the precipitated product was filtered off thro' a Gooch crucible and washed with the standardised minimum quantities of absolute alcohol (1 c.c.) and dry ether (1 c.c.) and then dried in a vacuum desiccator.

Yield 0.16 gm.

The alkali combined was determined by direct titration of a weighed quantity (0.158 gm.), dissolved in water, with $\frac{N}{10}$ H₂SO₄ (5.83 c.c.).

$$\% \text{-KOH combined} = \frac{56.1 \times 5.83 \times 0.10 \times 100}{1000 \times 0.158} = 20.7\% \text{-KOH.}$$

Further results are quoted in Table I (p.35).

B. SECOND METHOD.

The addition compound in this case was isolated by the simultaneous deacetylation and compound formation of the tetracetyl β -methylglucoside with a suitable quantity of potassium hydroxide.

Recrystallised tetra-acetyl β -methylglucoside (1.0 gm.) was dissolved in absolute alcohol (20 c.c.) and to this sufficient standard alcoholic potassium hydroxide solution was added to allow deacetylation and compound formation to take place and the additive complex precipitated by the addition of dry ether (100 c.c.). The flask was then corked and set aside for 15 minutes. The produce was filtered off through a Gooch crucible and washed as rapidly as possible with the standardised minimum quantities of absolute alcohol (1 c.c.) and dry ether (1 c.c.) to remove adhering potash solution and quickly transferred to a vacuum desiccator.

The alkali content of a sample of the addition complex was determined by titration of a weighed quantity (0.410 gm.), dissolved in water, against $\frac{N}{10}$ H_2SO_4 (15.30 c.c.) and phenolphthalein indicator.

$$\% \text{-KOH combined} = \frac{56.1 \times 15.30 \times 0.10 \times 100}{1000 \times 0.410} = 20.9\% \text{-KOH}$$

A series of similar experiments were conducted over a large range of alkali concentrations and the results obtained are tabulated in Table II (p.35).

A TYPICAL PREPARATION OF β -METHYLGLUCOSIDE
POTASSIUM HYDROXIDE.

To an absolute alcoholic solution (350 cc.) of tetra-acetyl β -methylglucoside (40 gm.) containing potassium hydroxide (40 gm.) dry ether (1500cc) was added till no further precipitation occurred. The mixture was shaken, corked and set aside for 30 minutes. The white precipitated product was then quickly filtered and washed as rapidly as possible with the minimum quantities of absolute alcohol (25 c.c.) and dry ether (25 c.c.) and then dried overnight over phosphorus pentoxide in a vacuum desiccator.

Yield 22 grams.

The product was a white, amorphous, very hygroscopic powder readily soluble in water.

The alkali content was determined by titration of a sample against $N/10$ acid.

Found 20.2 %

calculated for a mono alkali addition compound

$C_7H_{14}O_6$, KOH

KOH 22.4 %

A TYPICAL METHYLATION OF β -METHYGLUCOSIDE
POTASSIUM HYDROXIDE.

The finely powdered potassium hydroxide compound (20 gm.) was placed in a flask fitted with a stirrer and quickly covered with dimethyl sulphate (160 c.c.) which had been previously neutralised with anhydrous potassium carbonate. The contents were then vigorously stirred while the flask was surrounded with a water bath which was maintained at 60°C. for 5 minutes and then quickly raised to 70°C. for 10 minutes. The powdery solid coagulated to a sticky mass which adhered to the walls of the flask towards the end of the second period of heating. After cooling under the tap the solid was removed and thoroughly washed free of dimethyl sulphate with acetone. The methylated syrup was then extracted with hot dry methyl alcohol (75 c.c.) and after evaporating down under reduced pressure to a suitable volume the solution was set aside for 4 hours to allow the deposition of potassium methyl sulphate. On removal of this, to the clear filtrate an excess of an alcoholic solution of potassium hydroxide (40 c.c. of 4 N. solution) was added followed by dry ether (800 c.c.) till no further precipitation occurred. The residue

(17 gm.) was washed with small quantities of absolute alcohol and dry ether and kept for future methylations. After ensuring that further addition of alcoholic potash did not precipitate any more addition complex, the solution was acidified with glacial acetic acid and after the removal of the precipitated potassium acetate, was evaporated to dryness at 40°C. under reduced pressure.

ACETYLATION OF THE METHYLATED SYRUP.

The dried mixture of potassium acetate and methylated sugar was acetylated by heating with a mixture of acetic anhydride (50 c.c.) and anhydrous sodium acetate (10 gm.) for 3 hours on a water bath maintained at 95°C. The acetylation mixture, when cold, was poured into cold water and completely neutralised with solid sodium bicarbonate. The methylated sugar was extracted by several treatments with chloroform (300 c.c.) and the extracts combined and dried over anhydrous sodium sulphate overnight. After filtration the solvent was removed at 40-50°C. under diminished pressure and a slightly coloured syrup was obtained. Purification was

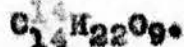
effected by distillation in a high vacuum. A clear syrup distilled over at 180-190°C. under 0.06 mm. pressure.

Yield 1.50 grams.

Analysis:

Found OMe 15.7%, CH₃CO 35.9%.

Calculated for a triacetylmethyl methylglucoside



OMe 18.6%, CH₃CO 38.6%.

$[\alpha]_D^{18^\circ C.} = -19.4^\circ$ (c = 0.91) in chloroform.

DEACETYLATION AND HYDROLYSIS OF SYRUP.

The syrup was deacetylated by the method of Zemplen (8). A cold chloroform solution (10 c.c.) of the syrup (1.1 gm.) was added to a cold dry methyl alcoholic solution (7 c.c.) of sodium (0.25 gm.) and after standing in cold water for a period of three hours the deacetylated syrup was extracted with water (10 c.c.) after acidification with glacial acetic acid. The chloroform extract was treated with more water (10 c.c.) and the combined aqueous extracts with more chloroform (5 c.c.). The aqueous extract was then

carefully evaporated down under diminished pressure on a water bath at 50°C. till the volume of the solution was reduced to suitable bulk. A test with Fehling's solution showed this solution to be non-reducing.

Hydrolysis was effected by means of 5% hydrochloric acid. Sufficient bench hydrochloric acid was added to make the resulting solution the desired strength and the mixture heated until the rotation of the solution became constant. This took 6 hours. The following readings were observed.

	$[\alpha]_D^{20^\circ}$		
0 mins.	+83.2°		
1 hour	69.4°	4 hours	54.8°
2 hours	61.1°	5 hours	53.9°
3 hours	56.4°	6 hours	53.0°

The solution was then neutralised with silver carbonate, filtered and, after very thorough washing of the silver residues with hot water and addition to the original extract, evaporated down under reduced pressure to a suitable volume. On evaporation colloidal silver appeared which was removed by filtration through a carbon bed. This solution was strongly reducing.

FORMATION OF THE OSAZONE.

To the clear aqueous solution (15 c.c.) of the hydrolysed syrup pure phenylhydrazine (0.8 c.c.) in glacial acetic acid (1.5 c.c.), crystalline sodium acetate (0.4 gm.) and a small quantity of sodium bisulphite (8) were added and heated on a water bath at 90°C. After some time the solution became opalescent and in 2 hours a precipitate had formed which was filtered off and after thorough washing with dilute acetic and water the product was dried in vacuo. The filtrate was heated for a further period when another crop of crude osazone was obtained and after washing was dried. Recrystallisation from aqueous alcohol gave a product suitable for analysis.

Yield * 0.05 grams.

Analysis:-

Found: OMe, 7.2%

calculated for a monomethyl glucosazone $C_{19}H_{24}O_4N_4$

OMe, 8.3%

m.p. 177-181°C. after several recrystallisations.

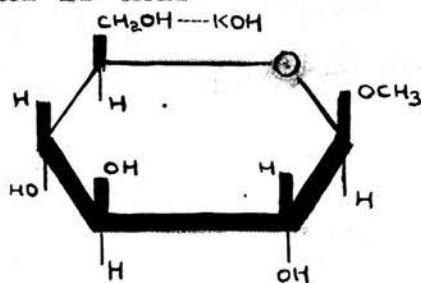
Identification of this methylated phenylosazone was obtained by means of mixed melting points with known specimens of 3-methyl glucosazone and 6-methyl glucosazone.

melting point of 3-methyl glucosazone 175°C.
mixed m.p. with 3-methyl glucosazone 160-163°C.
melting point of 6-methyl glucosazone 180-182°C.
mixed m.p. with 6-methyl glucosazone 181-182°C.
mixed m.p. of 6 methyl and 3-methyl
glucosazone 162-163°C.

From the evidence then it was concluded that the osazone isolated was a specimen of 6-methyl glucose phenylosazone.

S U M M A R Y .

1. By the addition of ether to an alcoholic solution of tetra-acetyl β -methylglucoside containing potassium hydroxide an alkali addition compound of β -methylglucoside was precipitated.
2. Direct analysis of the addition products indicated that one molecule of potassium hydroxide was associated with one molecule of β -methylglucoside.
3. By controlled methylation followed by acetylation a triacetyl monomethyl β -methylglucoside in 4% yield was obtained.
4. On treatment of the monomethylglucose derived from this a monomethyl glucose phenylosazone was obtained which was identified as 6-methyl glucosazone.
5. From the above results it was concluded that the potassium hydroxide residue was associated with the hydroxyl of the terminal primary alcoholic grouping in the β -methylglucoside molecule. The structure of the addition compound is thus



B I B L I O G R A P H Y.

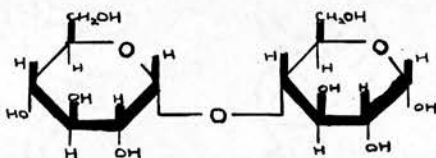
- (1). Percival J.C.S., 1934, 1160.
- (2). Percival J.C.S., 1935, 648.
- (3). Percival and Ritchie J. C.S., 1936, 1765.
- (4). MacKenzie and Quin J.C.S., 1929, 951.
- (5). Heddle this thesis p.1.
- (6). Koenigs and Knorr Ber., 1901, 34, 962.
- (7). Zemplén and Kunz Ber., 1923, 56, 1705.
- (8). Hamilton J.A.C.S., 1934, 56, 487.

PART III.

**THE ADDITION COMPOUNDS OF MALTOSE
AND POTASSIUM HYDROXIDE.**

DISCUSSION.

Attention was next directed towards the disaccharide maltose since it has the structure of an 4- α -glucosido glucose. The fact that this sugar is a disaccharide makes a study of its behaviour towards potassium hydroxide a much more complicated problem than in the case of the monosaccharides by reason of the two glucopyranose units capable of substitution.



Maltose.

A thorough search of the literature showed that there had been very little work done on the interaction of the alkali hydroxides with maltose.

Evans and Benoy (1) in a series of papers on the mechanism of carbohydrate oxidation showed that maltose in aqueous potash solution was subjected to two kinds of decomposition - enolic splitting and hydrolysis, but did not suggest any preliminary compound formation.

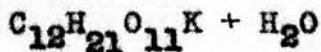
Hirsch and Schlags (2), who approached the subject from a physico-chemical point of view, regarded the sugars as weak acids and carried out a series of conductivity measurements in alkaline solutions. They

found in the various sugars examined evidence for the presence of more than one acidic grouping in the sugar molecule, the simplest assumption being that they were dibasic. The dissociation constants for maltose, the most "acid" sugar examined, were quoted for 25°C.

	$k_1 \times 10^{-13}$	$k_2 \times 10^{-14}$
Dissociation constant } for maltose	11.6	7.70

It is, of course, to be expected that the alkali will attach itself to the centres of maximal acidity in the molecule.

Herzfeld (3) in a description of the derivatives of maltose indicated the existence of a soda maltose complex to which he gave the formula, $C_{12}H_{21}O_{11}Na$. Whilst Lippman (4) showed the existence of compounds formed between potassium and sodium hydroxides and maltose and pointed out the importance of an alcoholic medium for their isolation. He gave the potash complex the formula



but mentioned no properties or gave any experimental details.

Percival (5), however, was able to show quite definitely by means of a titration method that alcoholic potash did combine with maltose and the results (Table I)

suggested that in the more concentrated alkali solutions three molecules of potash were apparently combined with one molecule of maltose and for weaker concentrations mixtures of compounds containing less potassium hydroxide were obtained. The structure of these compounds, however, was not further investigated.

The first step was to determine approximately the alkali combining capacity of maltose at higher concentrations than those quoted in this paper.

The white addition compound was immediately precipitated when standard alcoholic potash was added to an aqueous-alcoholic standard solution of maltose. After standing for 15 minutes the addition complex was filtered off and by titration(I) with acid of an aliquot part of the filtrate the amount of alkali removed from the solution was determined. Direct titration (II) of the compound, after washing with the minimum quantity of absolute alcohol to remove adhering potash solution, gave the amount of alkali associated directly. (see TABLE I. p. 57.) The variation between the results of the two methods is due to the decomposition by washing with alcohol of the higher addition compounds, and helps to illustrate the instability of these more complex derivatives. For a compound of the type $C_{12}H_{22}O_{11} \cdot 2KOH$, 100 gm. of maltose would require 32.8 gm. potassium hydroxide and for $C_{12}H_{22}O_{11} \cdot 3KOH$ 49.0 gm. alkali.

TABLE - I.

Maltose-potassium hydroxide.

Result No:-	1	2	3	4	5	6
total concentration of maltose - %	1.20	1.01	0.90	1.50	1.60	2.10
concentration of potassium hydroxide (normality)	Initial 1.370	1.159	1.00	0.92	0.40	0.15
	Final 1.360	1.150	0.92	0.80	0.31	0.04
%-age potassium hydroxide in isolated product.	Method I 32.2	30.9	30.8	31.3	26.6	22.2
	Method II 32.3	38.8	26.9	27.0	26.5	20.1

Calculated for $C_{12}H_{22}O_{11} \cdot 3KOH$, KOH , 35.0%

or 100 gm. maltose would combine with 49.0 gm. potassium hydroxide.

Calculated for $C_{12}H_{22}O_{11} \cdot 2KOH$, KOH , 24.7%

or 100 gm. maltose would combine with 32.8 gm. potassium hydroxide.

The results (Nos. (1-2), in conjunction with those quoted by Percival (Nos.3-6) show that one molecule of maltose is capable of uniting with one, two or three molecules of potash depending entirely on the concentration of the latter. The higher concentrations apparently favour the formation of the trialkali associated compound whilst at lower concentrations of alkali mixtures are produced.

As before the method adopted for the investigation of the structure of those compounds was to introduce a stable group in place of the loosely bound alkali. This was effected by mild methylation. Then by acetylation, deacetylation and hydrolysis and subsequent reacetylation, fractional distillation gave rise to di- and mono-methylated glucoses which were identified by further analysis.

The addition compound was subjected to mild methylation (5) with dry neutral dimethyl sulphate for a short time and after the extraction of the methylated syrup with alcohol, the unchanged maltose was eliminated by precipitation by alcoholic potash. By acetylation of the remainder a methylated maltose acetate was obtained in 10% yield calculated on the quantity of maltose initially present. This product was non-reducing showing

that one methoxyl residue was glucosidic. Deacetylation and hydrolysis was followed by partial fractionation by means of potassium hydroxide in an attempt to remove any free glucose as an insoluble addition compound. Acetylation of the non-precipitated portion yielded a syrup which was fractionated by distillation in a high vacuum. In this way a separation of the monomethyl and dimethyl glucose acetates was effected and both were subjected to a rigorous examination in order to determine the positions of substitution.

Under no conditions was it possible to isolate a dimethyl osazone from the dimethyl glucose triacetate fraction. This fact in conjunction with the identification of the monomethyl osazone obtained, as 6-methyl-glucosazone, indicated the structure of the acetate syrup of this fraction to be 2:6 dimethyl glucose triacetate. Further evidence as to the nature of the substitution was obtained from an examination of the lactone which was isolated by the deacetylation of the dimethyl acetate and, after cautious oxidation with bromine water, regulated heat treatment of the product. From the slow rate of fall of positive rotation (40.8° to 30.5° in seven days) it was identified as belonging to the " δ " -series, and from this it was concluded that position "4" in the molecule was not substituted. Further information as to the nature of the

substitution in the dimethyl fraction was furnished by the following method. Weerman (10,11) found that by subjecting the amides of α -hydroxy acids to treatment with alkaline sodium hypochlorite and semicarbazide hydrochloride, hydrazodicarbonamide was formed. Since this product is obtained in good yield and is readily identifiable, this method forms a useful and delicate test for determining the presence of free hydroxyl in the "2" position. This test was applied to a sample of the amide obtained from the lactone. Under conditions which proved to be the most suitable for gluconamide the amide of the acid under investigation failed to give any precipitate.

The syrup analysing as a monomethyl glucose tetraacetate was next studied. As was seen in the cases of cellobiose (12), sucrose (13), α and β -methylglucosides (14) it was expected that substitution would occur in the primary alcoholic residue of the monomethyl fraction, owing to this position being most open to attack. Repeated attempts to obtain 6-methyl glucosazone in this case were without success and the later identification of the phenylosazone as glucosazone showed, since the methyl group had been eliminated by the phenylhydrazine residue, that the substitution must have occurred at position "2" in the glucose molecule. The slow

mutarotation of the lactone obtained on oxidation (50.8° to 33.9° in one week) indicated that it readily produced a "γ" lactone so that position "4" was apparently unsubstituted. Unfortunately no crystalline derivatives of 2-methyl glucose could be obtained. Further confirmation was obtained by the treatment of the amide, derived from the lactone, with alkaline hypochlorite and semicarbazide hydrochloride (10,11). No precipitation whatever, was obtained, even on nucleation with a known crystal of hydrazodicarbonamide and under conditions which proved the most suitable for gluconamide. From these facts, in conjunction with the evidence derived from the osazones, the deduction is made that the monomethylated syrup obtained on the fractionation of the acetate was 2-methylglucose tetraacetate.

From a preliminary acetylation of the precipitated product after filtration of the precipitate, obtained on the addition of ether to the alkaline alcoholic extract of the methylated sugar in an attempt to remove all unchanged maltose, immediately after the treatment with dimethyl sulphate, it was apparent that a partially methylated substance had been removed from the solution. It was decided to investigate this acetylated syrup with

a view obtaining further evidence as to the nature of the composition of the alkali addition compounds of maltose. By the combination and acetylation of a number of precipitates from the same stage in later experiments the resulting mass was hydrolysed, followed by the addition of excess of alcoholic potash to remove free glucose, and subsequently acetylated. Fractionation of the acetylated product led to two distinct fractions, as before. These were a dimethyl glucose triacetate and a monomethylglucose tetra-acetate. The dimethyl product gave rise to a monomethyl osazone, 6-methyl glucosazone, and the monomethyl fraction to glucosazone. These results agree with those obtained from the main portion of the syrup.

From the foregoing facts, therefore, the deduction is made that one molecule of maltose is capable under the most favourable conditions of combining with three molecules of potassium hydroxide. Although in general a mixture of $C_{12}H_{22}O_{11} \cdot 3KOH$ with $C_{12}H_{22}O_{11} \cdot 2KOH$ and possibly $C_{12}H_{22}O_{11} \cdot KOH$ is produced. It is clear that one of the potassium hydroxide residues is attached to the reducing group, and since a dimethyl glucose, apparently 2:6 dimethyl glucose has been isolated it is suggested that in the non-reducing glucose residue, potassium

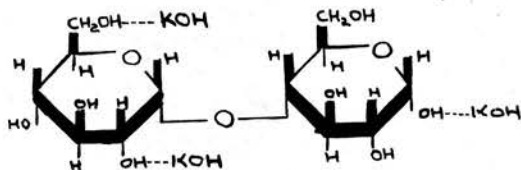
hydroxide residues are associated with the hydroxyl groups at positions 2 and 6. It is admitted at present that there is no way of distinguishing between the two glucose units but it is thought improbable that one glucopyranose unit could carry three potassium hydroxide residues. Furthermore in the case of lactose (12) definite evidence is available that the galactopyranose residue corresponding to the non-reducing glucose residue carries two of the three potassium hydroxide molecules in the corresponding lactose derivative $C_{12}H_{22}O_{11} \cdot 3KOH$. (although the KOH residues are associated with the positions 2 and 4 in this case). It would have been expected that the monomethyl glucose derived from $C_{12}H_{22}O_{11} \cdot 2 KOH$ would be a mixture of the 2- and 6-methyl glucoses, but no evidence whatever for the presence of 6-methyl glucose could be obtained despite a careful search amongst the osazones derived from the monomethyl glucose fragment. If the previous conclusions are correct therefore the dipotassium hydroxide maltose will have potassium hydroxide residues associated with the reducing group and position 2 of one of the glucopyranose residues, presumably the second. This is quite different from the case of cellobiose (12) which differs from maltose only in that the linkage between the glucopyranose units is β instead of α , for in this case

the reducing group and a primary alcoholic residue only are concerned. From the results obtained from α -methylglucoside it might have been considered more likely that the dipotassium hydroxide compound of maltose would have the second potassium hydroxide residue associated with the primary alcoholic residue as in the corresponding cases of β -methylglucoside and cellobiose. This is not the case however and position 2 seems more significant than position 6. This was also found to hold in the later work on amylose and cellulose. It is to be noted, however, that the tripotassium hydroxide compound of both maltose and lactose involve position 2.

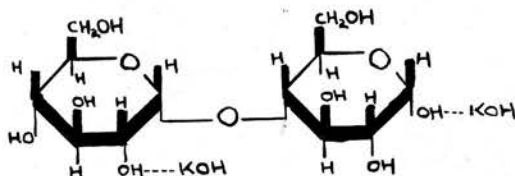
When this work was instituted it was hoped that the partial methylation of these potassium hydroxide derivatives would lead to crystalline mono- and dimethyl sugars and their derivatives. This hope has not been realized since the only crystalline derivatives isolated have been the osazones. Failure in this respect may be due to the production of equilibrium mixtures of α and β forms and even of ketoses due to the use of alkali at various stages. It has been necessary therefore to depend for identification on osazone formation and such experiments as lactone formation, the Weerman reaction, etc., and although the results are regarded as conclusive it is a matter for regret that more concrete evidence cannot be presented.

65.

The structure of the tripotash compound of maltose is



and that of the dipotash compound



EXPERIMENTAL.

—————

DETERMINATION OF THE COMBINED ALKALI.

Maltose (0.2042 gm.) was dissolved by gentle warming in a known volume (2.0 c.c.) of 80% alcohol. Cold standard alcoholic potassium hydroxide (15.0 c.c.) was added and the mixture allowed to stand for 15 minutes in a corked flask. The precipitated addition compound was removed by filtration through a sintered glass crucible.

- (a). An aliquot portion of the filtrate was treated with standard acid using phenolphthalein as indicator.
- and (b). the precipitate, after draining, was washed with the standardised minimum quantity (2 c.c.) of absolute alcohol to remove adhering potash solution and then dissolved in water and titrated with standard acid.

RESULTS:-

I. INDIRECT

Normality of the alcoholic potash employed was 1.545N.

But total volume of solution was 17.0 c.c.
Hence INITIAL NORMALITY of potassium hydroxide in the reaction solution was 1.370 N.

After standing for 15 minutes in a stoppered flask the reaction mixture was filtered.

2.0 c.c. filtrate require for neutralisation
 13.20 c.c. $N/5$ acid Hence FINAL NORMALITY of
 potassium hydroxide in the reaction solution was
 1.360 N.

Hence amount of alkali withdrawn from the reaction
 solution by 0.2042 gm. maltose was equivalent to
 to 17.0 c.c. of $1.370 - 1.360 = 0.010$ N. potash

*. 100 gm. maltose would withdraw

$$\frac{17.0 \times 0.010 \times 56.1 \times 100}{1000 \times 0.2042}$$

that is 46.7 gm. potassium hydroxide.

II. DIRECT

8.30 c.c. of $N/5$ acid were required for neutral-
 isation of the alkali of the dissolved precipitate.

*. 100 gm. maltose would combine with

$$\frac{8.30 \times 0.206 \times 56.1 \times 100}{1000 \times 0.2046}$$

that is, 46.8 gm. potassium hydroxide.

The results of a series of similar titrations are
 tabulated in Table I. (p. 57.).

A TYPICAL PREPARATION OF POTASSIUM HYDROXIDE MALTOSÉ.

Maltose (30 gm.) was dissolved in water (70 c.c.)
 by gentle warming till all was in solution. Alcohol
 (500 c.c.) was added until precipitation of maltose

began to take place and the solution was then mixed with alcoholic potash (300 c.c. of 0.7 N.). A copious white precipitate was formed and after standing for an hour was filtered off rapidly at the pump, washed quickly with the minimum quantities of absolute alcohol and dry ether and dried over phosphorus pentoxide in a vacuum desiccator overnight.

Yield 36 grams.

The product was a white, amorphous, very hygroscopic powder which tended to become yellow on keeping.

Analysis:—

By titration against $N/10$ H_2SO_4 and phenolphthalein indicator the alkali content was found to be

KOH, 29.1%

calculated for $C_{12}H_{22}O_{11}$, 3KOH. KOH, 33.0%.

$C_{12}H_{22}O_{11}$, 2KOH. KOH, 24.7%.

A TYPICAL METHYLATION OF MALTOSE POTASSIUM HYDROXIDE.

Methylation of the addition compound by means of dry methyl sulphate previously neutralised with potassium carbonate followed. The finely powdered maltose potassium hydroxide was put into a flask, fitted with a stirrer, and to this, neutral methyl sulphate (100 c.c.)

was quickly added. The flask was surrounded by a water bath maintained at 65°C. for five minutes and then at 70 - 75°C. for ten minutes, the contents being constantly stirred mechanically. It was noticed that towards the end of the second period of heating the methylation reaction mixture coagulated to a viscous mass which adhered to the walls of the flask. After cooling in ice the supernatant liquid was decanted off and the residue well washed with acetone to remove the adhering reactant, then the methylated product was extracted several times from the residual mass by means of hot dry methyl alcohol (150 c.c.). On cooling, the solution deposited crystals of potassium methyl sulphate which were removed by filtration at the pump and the filtrate was evaporated at 40°C. under reduced pressure to about one third of its original volume.

When cold, excess of alcoholic potash (100 c.c. of N.) was added to remove the unchanged maltose (I) from the sphere of reaction. A further quantity (II) was obtained by the addition of dry ether (300 c.c.). These dark coloured addition products were filtered off and after washing with small quantities of absolute alcohol and dry ether, were dried in vacuo and investigated. After ensuring that further addition of alkali caused no

more precipitation, the ethereal filtrate was acidified with glacial acetic acid and evaporated to dryness at 45°C. under reduced pressure. The product was a pale yellow syrupy mass (III) consisting of the methylated maltose and potassium acetate.

EXAMINATION OF THE PRECIPITATES.

The precipitates obtained with KOH were dissolved (separately) in water and carefully neutralised with dilute acetic acid and taken to dryness. Acetylation with pyridine and acetic anhydride gave, after the usual working up, small quantities of dark coloured syrups.

Methoxyl determinations revealed the presence of a considerable methoxyl content in the acetate syrup derived from the second (II) precipitate.

Precipitate	I	II
Methoxyl content) of acetate syrup)	0.74%	8.31%

By the mass acetylation of several of these precipitates obtained in former experiments at the same stage a quantity of syrup sufficient for a detailed analysis was obtained and examined later (p. 93).

ACETYLATION OF THE SYRUP. - III.

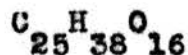
The syrupy mass was then acetylated by heating for three hours with a mixture of acetic anhydride (50 c.c.) and anhydrous sodium acetate (9 gm.) on a water bath maintained at 95°C. - 100°C. When cold the acetylation mixture was poured into cold water (300 c.c.) and completely neutralised with sodium bicarbonate. After filtering, the filtrate was thoroughly extracted with chloroform (500 c.c.) and the residue was washed with warm water and similarly treated. The combined extracts were dried by standing over anhydrous sodium sulphate and then evaporated to dryness under reduced pressure on a water bath at 45°C. The resulting product was a viscous, slightly discoloured syrup showing no reducing properties towards Fehling's solution.

Yield 3.0 grams.

Analysis:-

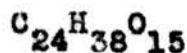
Found: OMe, 16.6%, CH₃CO, 35.1%

Calculated for a dimethyl pentacetyl methylmaltoside



OMe, 15.7%, CH₃CO, 36.2%

Calculated for a trimethyl tetra-acetyl methylmaltoside



OMe, 21.9% CH₃CO, 30.4%

$\left[\alpha \right]_{\text{D}}^{18^\circ} + 103^\circ$ in chloroform (c = 0.54)

DEACETYLATION AND HYDROLYSIS OF THE SYRUP FROM III.

The syrup was deacetylated by the method of Zemplén (15). The acetylated syrup (2.5 gm.) was dissolved in dry chloroform (10 c.c.) and cooled in ice and then added to a similarly cooled dry methyl alcoholic solution (10 c.c.) of sodium (0.5 gm.). The mixture was kept immersed in a basin of cold water for three hours and at the end of this period a slight excess of acetic acid was added. Extraction with water (20 c.c.), followed by addition of chloroform (10 c.c.) to the aqueous extract and water (20 c.c.) to the chloroform extracts. The aqueous extracts were combined and sufficient dilute sulphuric acid was added to make the resulting solution 1.5 N. This hydrolysis mixture was then heated on a water bath at 95°C. for five hours. Neutralisation with barium carbonate and thorough washing of the residue with warm water resulted in a yellowish coloured solution which was then evaporated to dryness under reduced pressure at 45°C. The last traces of moisture were removed with the aid of a benzene-alcohol mixture. The products of the hydrolysis of the deacetylated sugar were obtained by the thorough extraction of the dried residue with warm absolute alcohol and then partially fractionated by addition of alcoholic potash till no further

precipitation (IV) occurred. Dry ether (200 c.c.) was added to bring about complete precipitation (V). These precipitates were washed with small quantities of alcohol and ether to remove adhering potash solution and dried in a vacuum desiccator over phosphorous pentoxide. The ethereal filtrate was acidified and after the removal of the potassium acetate was taken to dryness at 40°C. under diminished pressure to yield a syrup (VI) mixed with potassium acetate (yield 2.1 grams.).

EXAMINATION OF THE PRECIPITATES.

The sugar alkali compounds when dry were dark in appearance and very hygroscopic. They were readily soluble in water.

Precipitate	IV.	V.
Yield	0.9 gms.	0.2 gms.

These were combined with the respective precipitates obtained in an earlier experiment and subjected to osazone formation. Phenylhydrazine (0.5 c.c.) was added to an acetic acid acidified aqueous solution of a small quantity (0.5 gm.) in both cases and the mixture heated on a water bath at 90°C. By the addition of a small quantity of sodium bisulphite the formation

of tarry oxidation products was avoided (16) and within 20 minutes' time a considerable quantity of osazone had appeared in the first case (IV.) and this was removed by filtration and additional crops were obtained on further heating. A small quantity of osazone was obtained in the second case (V) after three hours' heating. Analysis showed that the osazone derived from the first addition product was glucosazone whilst that derived from the second appeared to be partially methylated and was probably a mixture of glucosazone with a methylated osazone. Recrystallisation was effected from aqueous alcohol.

Analysis:-

Precipitate	I.	II.
m.p.	201°C.	151-160°C.
CH ₃ O	nil	3.0%

Further analysis of the osazone derived from the second alkali precipitate (II) was not possible owing to the small quantity available. On admixture with a known specimen of glucosazone the osazone derived from the first precipitate showed no depression in the melting point and hence was considered identical.

ACETYLATION OF SYRUP VI.

To the yellowish coloured mass obtained on evaporation of the ethereal filtrate after the partial separation of the products of hydrolysis, crystalline

sodium acetate (6 gm.) and acetic anhydride (50 ccs.) were added and the reaction mixture kept at 95-98°C. for three hours. When cold the acetylation mixture was poured into cold water (200 c.c.) and neutralised with solid sodium bicarbonate and extracted with chloroform (150 c.c.). After drying over anhydrous sodium sulphate the chloroform extract was evaporated to dryness under reduced pressure.

Yield 1.3 grams.

Analysis:-

Found: OMe, 15.9% CH₃CO, 37.1%

calculated for a dimethyl triacetyl glucose C₁₄H₂₂O₉.

OMe, 18.6%, CH₃CO, 38.5%

$\left[\alpha \right]_{D.}^{19^{\circ}C.} + 52.3^{\circ}$ in chloroform (c=0.8)

FRACTIONATION OF THE ACETYLATED SYRUP.

By the combination of syrups (7.4 gm.) at this stage from four parallel experiments having closely similar analyses a sufficient quantity was obtained to allow of a separation by distillation. The fractionation was effected by distillation in a high vacuum, the operation being carried out as rapidly as possible in order that the syrup might be exposed to the

destructive action of high temperatures for the minimum of time. Careful separation of the distillates resulted in four fractions being collected between 140° and 220°C. The residual discoloured mass and the syrup adhering to the walls and side tube of the distilling flask were treated with several small quantities of boiling chloroform and when cold, filtered. The extract was evaporated down and when all traces of solvent were removed was subjected to redistillation. This gave a further fraction (E). The separation effected is shown below.

Fraction	Temperature	Pressure	Yield	Methoxyl	$\left[\alpha \right]_{D.}^{20^{\circ}C.}$
No:-	in °C.	in mms.	in gm.	%-CH ₃ O.	in CHCl ₃
A.	140	0.10	0.06	10.6	-
B.	141-159	0.05	0.75	23.0	-
C.	160-180	0.04	4.08	17.5	+59.2
D.	181-220	0.05	1.48	10.0	+61.0
E.	205-225	0.07	0.23	9.27	-

These fractions were then examined in detail.

FRACTION - A.

This fraction was obtained as a slightly yellowish coloured syrup and probably contaminated with impurities of low boiling points. The very poor yield prevented a detailed analysis.

FRACTION - B.

The syrup obtained in the second fraction (0.75 gm.) was clear and colourless and appeared to be a mixture of the tri- and di-methylated acetates from the methoxyl result. Found: OMe, 23.0%.

calculated for a trimethyl diacetyl glucose $C_{13}H_{22}O_8$

OMe, 30.4%.

calculated for a dimethyl triacetyl glucose $C_{14}H_{22}O_9$

OMe, 18.6%.

By distillation a separation was made.

	Temperature. °C.	Pressure. gms.	Yield. gms.	Methoxyl. %
Fraction I.	A. 148	0.05	0.07	24.8
II.	B. 148-160	0.05	0.65	16.3

Methoxyl determinations on the products gave the values quoted in the table. Further analysis of the more highly methylated product was not possible owing to the poor yield. The other was combined with the third fraction.

FRACTION - C.

This third fraction (4.08 gm.) was redistilled and the resulting product, a clear, nearly colourless, viscous syrup, was subjected to a rigorous examination.

Analysis:-

Found: OMe, 17.5%, CH₃CO, 38.2%.

calculated for a dimethyl triacetyl glucose C₁₄H₂₂O₉

OMe, 18.6% CH₃CO, 38.5%.

$\left[\alpha \right]_{D.}^{18^{\circ}C.} + 59.2^{\circ}$ in chloroform (c = 0.46.).

A portion (0.67 gm.) of the syrup was deacetylated by Zemplén's (15) method. To the cold chloroform solution (5 c.c.) of the syrup a cold dry methyl alcoholic solution (5 c.c.) of sodium (0.15 gm.) was added and after standing 2½ hours in a basin of cold water was worked up in the usual manner. Pure phenyl-hydrazine (1.0 c.c.) in glacial acetic acid (2.0 c.c.) and sodium acetate (0.5 gm.) were added to the aqueous extract (16 c.c.) containing the deacetylated sugar and the whole heated on a water bath at 100°C. for a considerable time. A small quantity of sodium bisulphite was added to minimise the formation of tarry oxidation products as recommended by Hamilton(16). The osazone obtained was filtered off, washed thoroughly with dilute acetic acid to rid it of all traces of phenylhydrazine and dried in vacuo overnight. The crude product was a reddish brown solid. (Yield 0.014 gm.). After one recrystallisation from aqueous alcohol the osazone was analysed.

A micromethoxyl determination gave CH_3O 6.90%
 calculated for a monomethylglucosazone $\text{C}_9\text{H}_{24}\text{N}_4\text{O}_4$
 CH_3O 8.3%

By several further recrystallisations from aqueous alcohol a product suitable for a melting point determination was obtained.

m.p. 179°C.

This osazone was identified by means of mixed melting points with known specimens of 3-methylglucosazone and 6-methylglucosazone.

3-methylglucosazone	175°C.
mixed melting point with 3-methylglucosazone	161°C.
6-methylglucosazone	179°C.
mixed melting point with 6-methylglucosazone	177°C.

From the evidence then it was concluded that the osazone isolated was a specimen of 6-methylglucosazone. No trace of the presence of a dimethyl osazone could be found.

Thus it appeared that from a dimethyl sugar a

monomethyl osazone had been obtained. As this fact establishes one of the methyl groups in the "2" position and as the other has been proved to occupy the "6" position it follows that the original acetate syrup must have been

2,6 dimethyl 1,3,4, triacetyl glucose.

Further evidence of the structure of this acetate was obtained later.

FORMATION OF THE LACTONE FROM THE
DIMETHYL GLUCOSE.

A portion of the acetylated syrup was deacetylated by means of barium hydroxide. To a weighed quantity (0.60 gm.) of the syrup dissolved in acetone (20 c.c.) twice the theoretical quantity necessary to neutralise the acetyl content (previously determined) of a saturated standardised aqueous solution of barium hydroxide was added and the mixture shaken at frequent intervals during 4½ hours, the experiment being conducted at room temperature. At the end of this period the barium was precipitated as sulphate by the careful addition of $N/10$ H_2SO_4 . By the passage of a stream of air through the solution heated on a water bath at $45^{\circ}C$. the acetone

was removed and the precipitate coagulated and the latter removed by filtration through a fine grain paper. The filtrate, when free of barium and sulphate ions was evaporated down at 35°C. till the volume became about 25 c.c. This solution was then oxidised with bromine (2.5 c.c.) by heating, firstly at 35°C. for 60 hours and secondly at 45°C. for 40 hours. Aeration of the solution removed the bromine and neutralisation was effected by the addition of silver carbonate. After filtration the silver salt of the sugar acid was decomposed by the passage of hydrogen sulphide the clear filtrate was taken to dryness under reduced pressure and then heated at 100°C./0.05 m.m. for three hours to bring about conversion to lactone. The product was a nearly colourless syrup.

Yield 0.23 grams.

Analysis:-

Found	OMe,	26.7%
calculated for a dimethyl gluconolactone $C_8H_{14}O_6$		
	OMe,	30.1%

0.098 gm. lactone required ~~1.45~~ c.c. 0.02 N. NaOH for complete neutralisation.

calculated for a dimethyl lactone $C_8H_{14}O_6$ 21.9 c.c.

0.2 N. NaOH

HYDROLYSIS OF THE LACTONE.

The mutarotation of an aqueous solution (10 c.c.) of the lactone (0.05 gm.) was observed to give the following results:-

TIME	READING	$[\alpha]_{D}^{18}$
5 mins.	+ 0.24	+ 48.9
10 mins.	0.20	40.8
20 mins.	0.18	36.8
40 mins.	0.18	36.8
1 hour	0.18	36.8
2 hours	0.18	36.8
21 hours	0.18	36.8
2 days	0.16	30.5
7 days	0.16	30.5 (constant)

Since equilibrium was only attained very slowly the lactone was considered to be a γ -lactone and hence the methyl group was not in position 4.

AMIDE FORMATION.

A quantity (0.20 gm.) of the lactone was then dissolved in anhydrous methyl alcohol (10 c.c.) and to the cold solution a methyl alcoholic solution of

ammonia saturated at 0°C. was added and kept at 0°C. for 24 hours. After removal of the solvent at room temperature under diminished pressure a hard, slightly coloured glass was obtained. Attempts at crystallisation were not successful.

Yield 0.15 grams.

Analysis:-

Found: OMe, 24.9% N, 6.0%.

calculated for a dimethyl gluconamide $C_8H_{17}O_6N$.

 OMe, 27.8% N, 6.28%

$[\alpha]_D^{19} + 51.8^\circ$ in water (c = 0.4)

A portion of the amide was subjected to Weerman's (11) alkaline hypochlorite test to determine whether or not the "2" position was substituted. To an ice-cold aqueous solution (1.0 c.c.) of the amide (0.1 gm.) a small quantity of a standard solution of recently prepared sodium hypochlorite (0.5 c.c. of a 6.9% solution) similarly cooled was added and set aside in an ice bath for three hours. Finely divided sodium acetate was then added followed by an excess of a saturated aqueous solution of semicarbazide hydrochloride. No precipitate was formed but after standing for 12 hours at zero a slight turbidity was apparent.

Control experiments with the same quantity (0.1 gm.) of gluconamide gave, after standing three hours at 0°C., 0.028 gm. hydrazodicarbonamide (m.p. 258°C.). The sensitivity of this test for the recognition of amides of the α -hydroxy acids was shown by the isolation of 0.004 gm. hydrazodicarbonamide from 0.010 gm. gluconamide.

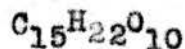
FRACTION D.

The reducing syrup (1.48 gm.) in this case was clear and colourless. It too was subjected to a rigorous examination.

Analysis:-

Found: OMe, 10.0%.

calculated for a monomethyl tetra-acetyl glucose



OMe, 8.57%

$[\alpha]_{D.}^{18^{\circ}C.} + 61.0^{\circ}$ in chloroform (c = 0.56)

It is apparent that complete separation from the dimethyl glucose had not been effected. Further vacuum distillation failed to effect a separation.

DEACETYLATION AND OSAZONE FORMATION.

Deacetylation was carried out by Zemplén's method (15). To an ice-cold chloroform solution (9.c.c.) of the syrup (0.71 gm.) a similarly cooled dry methyl alcoholic solution (8 c.c.) of sodium (0.15 gm.) was added and after standing three hours in a basin of cold

water was acidified with acetic acid. The deacetylated sugar was obtained by extraction with water in the usual manner. Phenylhydrazine (1.0 c.c.) and a small quantity of sodium bisulphite (6) were added to the aqueous extract and the whole heated on a water bath at 90°C. In an hour a precipitate had formed and after removal a second crop was obtained on further heating. These precipitates were well washed with dilute acetic acid and water and dried in a desiccator.

Yield 0.16 grams.

Analysis:-

The sample used for analysis was recrystallised twice from aqueous alcohol.

OMe,	nil.
m.p.	199°C.

On admixture with an authentic specimen of glucosazone the melting point showed no depression and hence was assumed identical.

LACTONE FORMATION.

Deacetylation was carried out by means of barium hydroxide. To a portion (0.40 gm.) of the syrup in

acetone (20 c.c.) twice the theoretical quantity, necessary to neutralise the acetyl content, of a saturated solution of barium hydroxide was added and after thorough shaking the mixture was set aside for 5 hours. The barium was then precipitated by the addition of the calculated quantity of $\frac{N}{10}$ H_2SO_4 and removed and the filtrate was shown to be free from barium sulphate ions. The solution was found to be reducing towards Fehling's. After evaporating down to a suitable volume (20 c.c.) the deacetylated sugar was oxidised by bromine (2.5. c.c.) by heating on a water bath at $40^\circ C.$ for 96 hours. At the end of this period this solution was shown to be non-reducing. By aeration of the solution the bromine was removed and neutralisation of the acid effected by the addition of an excess of pure silver carbonate. After filtration, by the passage of a stream of hydrogen sulphide through the liquid, the silver salt of the sugar acid was decomposed. The silver sulphide was filtered off and the filtrate taken to dryness under reduced pressure. Conversion to the lactone was brought about by heating under 0.05 m.m. pressure on a boiling water bath. The resulting product was a colourless

syrup.

Yield 0.23 grams.

Analysis:-

Found: OMe, 14.3%

calculated for a monomethylgluconolactone
 $C_7H_{12}O_6$.

OMe, 16.1%.

0.041 gm. lactone required 3.71 c.c. 0.02 N. NaOH for
 complete neutralisation.

calculated for a monomethyl lactone $C_7H_{12}O_6$ 9.76 c.c.

0.2 N. NaOH.

HYDROLYSIS OF THE LACTONE.

The lactone (0.036 gm.) was dissolved in water (10 c.c.) and the change in rotation followed polarimetrically.

TIME	READING	$[\alpha]_{\lambda=D}^{19^{\circ}\text{C.}}$
20 mins.	0.18	+ 50.8
40 mins.	0.18	50.8
1 hour	0.18	50.8
2 hours	0.15	42.4
4 hours	0.12	42.4
24 hours	0.12	33.9
72 hours	0.12	33.9
150 hours	0.09	26.4
1 week	0.09	26.4 (constant)

That constancy of rotation was attained only very slowly indicated the presence of a " γ " lactone.

AMIDE FORMATION.

The amide was obtained in good yield by adding a cold methyl alcoholic solution (5 c.c.) saturated with ammonia at 0°C. to a dry methyl alcoholic solution (10 c.c.) of the lactone (0.18 gm.) and, after standing at 0°C for two days, removing the solvent by evaporation in a vacuum desiccator. The product was a hard opaque glass. Attempts at crystallisation were not successful.

Yield 0.13 grams.

Analysis:-

Found: OMe, 10.8%, N, 16.38%

calculated for a monomethyl gluconamide $C_7H_{15}O_6N$.

OMe, 14.8%, N, 16.70%

$\left[\alpha \right]_{D.}^{19^{\circ}C.} + 37.2^{\circ}$ in water (c = 0.61)

To a portion (0.08 gm.) of the amide dissolved in water (0.20 c.c.) sodium hypochlorite (0.30 c.c.) containing 69 gm. NaOCl/litre was added and after thorough mixing was kept at zero for three hours. Finely divided sodium acetate was then added until no more would dissolve followed by excess of a saturated solution of semicarbazide hydrochloride. No precipitation was observed. On nucleation with a known crystal of hydrazodicarbonamide no precipitation occurred on standing overnight under the most suitable experimental conditions. From this result it was assumed that the " α " or "2" position in the molecule was substituted.

ATTEMPTED ISOLATION OF 2-METHYLGLUCOSE PHENYLHYDRAZONE.

To show further that the second methyl group was attached to the second carbon atom grouping an attempt was made to isolate the corresponding phenylhydrazone (7, 19).

After deacetylation of the syrup (0.39 gm.) the aqueous extract containing the deacetylated sugar was carefully evaporated to dryness. The residue, dissolved in water (0.5 c.c.), was allowed to stand at room temperature for 12 hours with phenylhydrazine (0.5 c.c.) and glacial acetic acid (1 drop). A general opaqueness of the solution occurred but no precipitation even at the end of 36 hours. Failure to obtain the phenylhydrazone was attributed to the use of too large a volume of water or to the presence of sodium acetate, previous experience (8) having shown that impure 2-methyl glucose does not readily yield the phenylhydrazone. The osazone, however, was isolated by the addition of water, a few drops of glacial acetic acid and a little sodium bisulphite to the solution and heating on a water bath at 95°C. for 50 minutes.

Yield 0.03 grams.

By recrystallisation twice from aqueous alcohol a bright yellow osazone was obtained which was readily identified as glucosazone.

A micromethoxyl determination showed absence of methoxyl

A melting point determination gave m.p. 198°C.

A mixed melting point with a known specimen of glucosazone showed no depression.

FRACTION E.

This last fraction was obtained by dissolving up the syrup left on the walls and side tube of the distilling flask, after the separation of the fourth fraction, in dry chloroform and subjecting the residue, after removal of the solvent, to re-distillation. The syrup distilled over within the range 205 - 225°C.

Yield 0.23 grams.

A micromethoxyl determination gave OMe, 9.27%
calculated for a monomethyltetracetyl glucose $C_{15}H_{22}O_{10}$

OMe, 8.57%

DEACETYLATION AND OSAZONE FORMATION.

The remaining portion (0.21 gm.) of the syrup was dissolved in warm chloroform (5 c.c.) and when ice-cold was added to an ice-cold solution of sodium (0.06 gm.) in dry methyl alcohol (5 c.c.) and set aside in a beaker of cold water for three hours. After acidification with glacial acetic acid the deacetylated sugar was extracted with water in the usual manner. To

the aqueous extract pure phenylhydrazine (0.6 c.c.) in glacial acetic acid (0.9 c.c.), sodium acetate (0.3 gm.) and a small quantity of sodium bisulphite (6) were added and heated on a water bath at 90°C. Within an hour a precipitate had appeared. This was removed, washed, and dried in vacuo. The residual solution was heated for a further period when slight additional precipitation occurred.

Analysis:-

The sample used for analysis was recrystallised three times from aqueous alcohol.

A micromethoxyl determination gave OMe, 0.98%.

A melting point determination was made m.p. 191-195°C. Owing to the small quantity of osazone obtained on recrystallisation it was impossible to isolate a better specimen. These results, however, were interpreted as supporting the previous deductions.

EXAMINATION OF THE SECOND PRECIPITATED PRODUCT OBTAINED ON THE ADDITION OF ALKALI AND ETHER TO THE ALCOHOLIC EXTRACT OF THE METHYLATED SYRUP.

It was decided to examine the precipitate (II - p) obtained on the addition of dry ether to the alcoholic potash solution of the methylated sugar subsequent to

the treatment with dimethyl sulphate. Three such precipitates, obtained from experiments made under similar conditions, were combined and subjected to a detailed analysis.

Precipitates	I.	II.	III.
Weight in gm.	8.0	14.0	8.0
KOH - %	-	4.3	1.3

The combined mass was dissolved in water and the alkali carefully neutralised with acetic acid and after taking to dryness under reduced pressure was acetylated.

Sodium acetate (2 gm.) and acetic anhydride (150 c.c.) were added and the reaction mixture kept at 95°C. for 2½ hours. When cold the acetylation mixture was poured into cold water (750 c.c.) and neutralised with solid sodium bicarbonate. Thorough extraction with chloroform (200 c.c.) followed and after drying over anhydrous sodium sulphate the extracts were evaporated to dryness under diminished pressure on a water bath at 40°C. giving a dark brown syrup.

Yield 5.10 grams.

Analysis:-

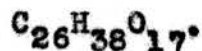
Found: OMe, 12.3%.

calculated for a dimethyl pentacetyl methylmaltoside



OMe, 15.6%

calculated for a monomethyl hexacetyl methylmaltoside



OMe, 9.98%.

HYDROLYSIS OF THE SYRUP.

Zemplén's (15) method of deacetylation was employed. To the cold chloroform solution (15 c.c.) of the syrup a cold solution of sodium (0.75 gm.) in dry methyl alcohol (10 c.c.) was added and the whole immersed in a basin of cold water for 3½ hours. At the end of this period sufficient acetic acid was added to neutralise the sodium and the deacetylated sugar extracted twice with water (30 c.c.) from the reaction solution. Sufficient sulphuric acid was added to make the resulting solution 1.5 N. and this was heated on a water bath at 95°C. for four hours - the time necessary for constant rotation to be attained. After neutralisation

of the acid with barium carbonate and thorough washing of the barium residues with hot water the clear solution was taken to dryness under reduced pressure. Last traces of water were removed with the aid of the benzene-alcohol mixture. The hydrolysed syrup was then extracted from the inorganic impurities by hot absolute alcohol and to these extracts, when cold, excess of alcoholic potash (2 N. solution) was added. After ensuring that further addition of alkali did not precipitate any more product dry ether was added to complete the precipitation. These addition products were filtered off, washed free of adhering potash solution with the minimum quantities of absolute alcohol and dry ether and dried over phosphorus pentoxide. The filtrate was acidified and after the removal of the precipitated potassium acetate was taken to dryness under diminished pressure at 40°C.

EXAMINATION OF THE PRECIPITATES.

The dried precipitated sugar alkali compounds were hygroscopic powders readily soluble in water.

Precipitate	I	II
Yield	0.32	0.14 grams.

I. was dissolved in water (25 c.c.) and after acidification with glacial acetic acid phenylhydrazine (0.5 c.c.) and sodium bisulphite were added and the mixture heated on a water bath at 90°C. After 25 minutes a precipitate had appeared and this was filtered off, washed thoroughly with dilute acetic acid and dried in vacuo over phosphorus pentoxide. Additional crops of osazone obtained on further heating were similarly treated.

Analysis:-

One recrystallisation from aqueous alcohol gave a bright yellow crystalline osazone.

A micromethoxyl determination gave OMe nil.

A melting point determination was made m.p. 201°C.

No depression of the melting point occurred on admixture with an authentic specimen of glucosazone and hence it was assumed identical.

The second precipitate (II) was similarly treated. An osazone was obtained within 45 minutes and when dry was recrystallised from aqueous alcohol.

Analysis:-

A micromethoxyl determination gave OCH_3 1.30%.

A melting point determination was made m.p. 183-186°C.

This was considered to be an impure specimen of glucosazone slightly contaminated with a methylated osazone.

ACETYLATION OF THE FILTRATE.

The residue obtained on evaporation of the acidified ethereal filtrate was acetylated. To the yellowish coloured mass anhydrous sodium acetate (5 gm.) and acetic anhydride (64 c.c.) were added and the mixture heated on a water bath maintained at 95°C. for 2 hours. When cold the reaction mixture was poured into water (350 c.c.) and after the usual working up a clear brown syrup was obtained.

Yield 0.71 grams.

Analysis:-

A macromethoxyl determination gave OMe 12.5%

calculated for a dimethyltriacyl glucose $C_{14}H_{22}O_9$

CH_3O 18.6%

calculated for a monomethyltetracyl glucose

CH_3O 8.57% $C_{15}H_{22}O_{10}$

FRACTIONATION OF THE ACETYLATED
SYRUP.

The acetylated syrup was transferred to a small distilling flask and after complete removal of solvent by heating at $100^{\circ}C.$ /0.5 m.m. for half an hour was subjected to fractional distillation. Two fractions were collected.

Fraction No:-	Temperature in $^{\circ}C.$	Pressure m.m.	Yield gm.	Methoxyl % - OMe	$[\alpha]_{D}^{18^{\circ}C.}$ in $CHCl_3$
I.	180-184	0.03.	0.322	16.4	+57.6 $^{\circ}$
II.	210-220	0.04	0.204	7.85	-

These fractions were then examined in detail.

FRACTION I.

The first fraction was a clear and colourless syrup.

Analysis:-

Found: OMe, 16.4%

calculated for a dimethyl triacetyl glucose $C_{14}H_{22}O_9$

OMe, 18.6%

$[\alpha]_{\lambda = D}^{18^{\circ}C.} + 57.6^{\circ}$ in chloroform ($c = 0.32$).

The syrup was deacetylated by the sodium methoxide method (15). To a cold chloroform solution (5 c.c.) of the syrup a cold methyl alcoholic solution (5 c.c.) of sodium (0.08 gm.) was added and set aside in a beaker of cold water for three hours. The solution was neutralized with acetic acid and the deacetylated sugar thoroughly extracted with water (20 c.c.). An osazone (0.006 gm.) was obtained by addition of pure phenylhydrazine (0.5 c.c.) in glacial acetic acid (0.3 c.c.) and sodium acetate (0.2 gm.) to the aqueous extract and heating at $90^{\circ}C.$ for $3\frac{1}{2}$ hours. After washing and drying in the usual way the product was recrystallised

from aqueous alcohol.

A melting point determination was made m.p. 171-175°C.

A micromethoxyl determination gave OMe, 7.31%.

calculated for a monomethylglucosazone $C_{19}H_{24}O_4N_4$

OMe, 8.3%.

FRACTION II.

An analysis of the syrup obtained in this fraction showed it to be monomethyl glucose acetate.

Found: OMe, 7.85%.

calculated for a monomethyl tetra-acetyl glucose

$C_{12}H_{22}O_{10}$.

OMe, 8.57%.

A cold dry methyl alcoholic solution (5 c.c.) containing a small quantity of sodium (0.05 gm.) was added to a cold chloroform solution (5 c.c.) of the syrup (0.18 gm.) and left for three hours in a basin of cold water. After acidification with acetic acid the deacetylated sugar was extracted with water (15 c.c.) in the usual manner.

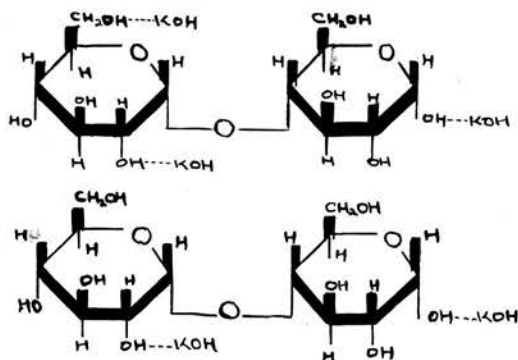
The aqueous extract was then treated with phenylhydrazine (0.6 c.c.) and sodium acetate (0.3 gm.) and heated at 90°C. In about one hour a precipitate had appeared and when filtered off the residual solution was heated for a further period when a second crop of osazone was obtained. After washing thoroughly with dilute acetic acid and water the osazone was dried in a vacuum desiccator. Recrystallisation was effected by means of aqueous pyridine to yield an osazone m.p. 198°C., OMe, nil, identical with glucosazone. This was confirmed by a mixed melting point determination.

S U M M A R Y.

1. Titration experiments indicated that maltose could form a compound in which three molecules of potassium hydroxide were associated with one molecule of maltose.
2. Mild methylation of the addition product gave rise to a mixture of partially methylated maltoses in 10% yield.
3. By deacetylation and hydrolysis, then reacetylation and fractional distillation a separation of the products of hydrolysis into di- and monomethylated glucose acetates was achieved.
4. The isolation of a monomethyl glucosazone from the dimethyl fraction, coupled with the identification of this osazone as 6-methyl glucosazone indicated the structure of the dimethyl compound to be 2:6 dimethyl glucose.
5. The isolation of glucosazone in good yield from the

monomethyl fraction showed position "2" to be substituted and indicated the structure of the monomethyl acetate syrup to be 2-methyl glucose.

6. Confirmation of the structure given was obtained from the rate of hydrolysis of the lactones obtained on oxidation and Weerman's reaction on the corresponding amides.
7. Treatment of the alkali addition precipitates obtained on the addition of dry ether to the alcoholic potash solution of the methylated sugar subsequent to treatment with dimethyl sulphate, in a similar manner, confirmed the previous results.
8. Evidence is therefore presented that 2-methylglucose and 2:6 dimethyl glucose are derived from the addition compounds. It is therefore concluded that the potassium hydroxide residues probably occupy these positions in the glucopyranose unit of maltose. The structure of the alkali addition compounds of maltose is then



B I B L I O G R A P H Y.

- (1) Evans and BenoyJ.A.C.S., 1930, 52, 294.
- (2) Hirsch and Schlags ..Z. physik. chemie, 1929, A,
141, 387.
- (3) HerzfeldAnn., 1883, 220, 206.
- (4) Lippmann "Chemie der Zuckerarten"
(1904), 1494.
- (5) PercivalJ.C.S., 1934, 1160.
- (6) HamiltonJ.A.C.S., 1934, 63, 2884.
- (7) Brigl and Schinle ...Ber., 1929, 62, 1716.
- (8) MunroThesis, Edin. Univ., 1936.
- (9) Brigl and Schinle....Ber., 1930, 63, 2884.
- (10) WeermanRev. trav. chim., 1918, 37,
16.
- (11) WeermanAnn., 1913, 401, 1.
- (12) Percival and
RitchieJ.C.S., 1936, 1765.
- (13) PercivalJ.C.S., 1935, 648.
- (14) HeddleThis thesis p. 10 and 32.
- (15) Zemplen and Kunz ...Ber., 1923, 56, 1705.

PART IV.

**THE ADDITION COMPOUNDS OF AMYLOSE
AND POTASSIUM HYDROXIDE.**

THE ADDITION COMPOUNDS OF AMYLOSE
AND POTASSIUM HYDROXIDE.

D I S C U S S I O N.

The next step in the elucidation of the structures of the alkali addition compounds of the carbohydrates was to investigate a polysaccharide built up from maltose residues, amylose, the water soluble constituent of starch.

The chemistry of starch and the other polysaccharides has occupied the attention of chemists for close on a century and although tremendous advances have been made yet the precise nature of many complicated polysaccharides remains unsolved. The reason for this is probably because of the tremendous difficulties experienced in the treatment of these substances as they fail to respond to the usual methods of attack which have proved so profitable in solving the compositions and structures of simpler compounds. No really notable advance was made till after final adoption of the pyranose ring structure for glucose when Haworth (1) and his co-workers put forward the view that the starch molecule was composed of glucose units joined together by ~~α~~ glucosidic linkages.

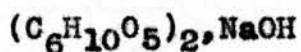
Nägeli (3) in 1858 was the first to show that starch consisted of more than one substance and later workers found that the granules of potato starch consisted of an outer coating of an insoluble substance known as "amylopectin" protecting an inner water soluble material known as "amylose". Schardinger (4) and later Pringsheim (5) were able to isolate from starch by bacteriological means a series of well defined amyloses some of which were crystalline and Karrer (6) was able to show that these amyloses were closely related to maltose.

A thorough search of the literature showed that very little work had been done on the alkali addition compounds of amylose. Samec (7) studied the action of alkalis upon starch during his work on the "Plant Colloids" from a physical chemical point of view. He found that the initial changes produced by alkalis was due to a reaction with the phosphoric acid grouping present in the starch molecule and that further action resulted in a combination with the groups in the starch molecule itself. Finally when the concentration of alkali was sufficiently strong peptonisation of the starch apparently occurred. Of the alkalis employed

Samec found potassium hydroxide to be the most reactive.

Liepatof (8) showed that the velocity of adsorption of alkali was very much greater from dilute solutions than from concentrated solutions.

In neither case was any attempt made to isolate the starch alkali compound and it was left to Karrer (9,10,11.) to show definitely that such existed. But owing to the fact that he conducted his experiments in the presence of water in which medium the addition complexes are readily decomposed little faith can be placed in his deductions. He suggested, and his analytical results supported the view, that there was a compound formed between amylose and potassium hydroxide in which one molecule of alkali was associated with one molecule of diamylose.



In later work Karrer (9,11) was able to show a similar relationship existed with potassium and barium hydroxides. Apart from analysing the compounds obtained he made no attempt to determine their structure and was apparently at a loss to explain the exact nature of the association between polysaccharide and alkaline hydroxide.

TABLE - I

Concn. of amylose - %	1.9%	1.3%	1.1%	.72%	.49%	.37%
Initial Normality	.532	.372	.277	.437	.592	.620
Concn. of KOH	.497	.268	.261	.385	.572	.605
Final Normality						
KOH in grams combined with 100 gms. amylose	Indirect method	27.7	27.2	28.3	26.5	30.0
	Direct method	24.5	23.4	21.9	25.1	29.7

For a compound $(C_6H_{10}O_5.KOH)_x$ 100 gm. amylose would combine with 34.6 gm. potassium hydroxide.

TABLE - 2

Initial normality	1.050	.996	.815	.775	.642	.481	.420	.300
Final normality	.770	.733	.446	.565	.540	.390	.247	.152
KOH in gms required to de-acetylate 100g.triacetate.	62.5	57.6	58.9	53.7	51.0	49.3	46.7	46.7
KOH in grams in Indirect combination with Direct 100 gms amylose method	27.1	27.2	27.1	29.1	27.1	27.0	28.9	35.4
	29.3	30.0	28.9	30.5	30.9	29.4	32.1	34.6

Calculated for $(C_6H_{10}O_5.KOH)_x$, 100 gm. amylose would combine with 34.6 gm. potassium hydroxide.

A series of titrations on similar lines to the cases studied previously was made with small quantities of amylose. But some difficulty was experienced in obtaining evidence as to the number of molecules of alkali combining with the amylose unit owing to the comparatively short period over which amylose, prepared by the method of Baird, Haworth and Hirst (12), would remain soluble in cold water. The results (TABLE - I) obtained suggested for the concentrations of alkali employed the presence of a compound in which one molecule of potassium hydroxide was associated with one molecule of amylose. Experimentally, a weighed quantity of amylose was dissolved in a certain volume of water and absolute alcohol added just to precipitation point. To this a measured volume (excess) of standard alcoholic potash was added. After standing for $2\frac{1}{2}$ hours with occasional agitation, titration of the filtrate by means of standard acid followed and the residue after washing with the standardised minimum quantities of absolute alcohol and dry ether to remove any adhering potassium hydroxide solution was dissolved in water and titrated with standard acid.

To determine whether a dialkali associated compound existed, as well as to confirm these preliminary

results, it was found necessary to prepare the acetate and by simultaneous deacetylation and compound formation prepare the addition compound and analyse it as was done by Percival and Ritchie (13) in their work on cellobiose. In this way the presence of water was excluded.

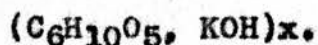
In the literature there are several methods for the preparation of amylose acetate. It has been found that if unmodified starch be employed acetylation by either ordinary or specialised means generally leads to such small yields that doubt may be raised as to whether the product is an acetylation derivative of the unchanged starch or is derived from a portion only of the polysaccharide which has been disaggregated during the process of acetylation. It is obvious then that the starch before acetylation must be subjected to some preliminary activating process in which the state of molecular aggregation is altered. Peiser (14) found that the preliminary precipitation of starch from an aqueous starch paste by alcohol yielded a product which was much more susceptible to acetylation than the original starch and which appeared to differ only in being more finely divided as a result of the bursting of the granules. Baird, Haworth and Hirst (12) however, subjected the starch granules to what appeared

to be a process of surface etching by heating an alcoholic hydrogen chloride suspension of starch and after filtration dissolving the product in boiling water and reprecipitating by alcohol. It is found that the first product retains a small amount of hydrogen chloride which cannot be removed by ordinary washing but after solution in boiling water the resulting precipitated product contains no hydrogen chloride and is devoid of reducing action towards Fehling's solution. The "prepared" starch too was quite soluble in cold water but after standing a few hours it reverted to the original insoluble form. In other words the process undergone has disaggregated the molecules temporarily and reaggregation to a higher complex takes place on standing.

Acetylation was readily effected by acetic anhydride in the presence of pyridine according to the method of Bergmann and Knehe (15). Another method (Haworth, Hirst and Webb, (16).) using acetic acid containing chlorine and acetic anhydride was employed but the resulting acetate required considerable purification and it was abandoned in favour of the pyridine method. Difficulty too was experienced in estimating the requisite quantity of chlorine. Acetylation by means

of sulphuric acid and acetic anhydride (17) is stated in the literature to give a 10% yield of inferior material.

From the acetate it was possible to investigate the alkaline combining capacity of amylose over a wide range of alkaline concentrations. Briefly the method is to add alcoholic potash to two weighed quantities of amylose acetate suspended in absolute alcohol and after standing for a short period to analyse one of these for the total amount of alkali used up by the two processes - deacetylation and compound formation, and the other for the amount required for deacetylation alone. The experimental details are given later in the "Experimental" section (p 126). This method has the advantage that the whole experiment is conducted in the medium of absolute alcohol thereby avoiding ambiguous results due to decomposition of the additive complex by water. From the results (Table No:-2.) it is evident that if we assume compound formation has taken place then only one molecule of potassium hydroxide associates with one molecule of amylose and this is shown by the formula



That is, 100 grams amylose will combine with 34.6 grams potassium hydroxide.

It was not possible to examine the behaviour at lower alkali initial concentrations than 0.3 N owing to the necessity of keeping the total reaction volume at a minimum and at the same time adding sufficient standard alkali to deacetylate the acetate and to have sufficient excess to allow of addition compound formation. At higher concentrations the solubility of potassium hydroxide in alcohol becomes the limiting factor. The gradual decrease in the quantity of alkali required for deacetylation is interesting. It should be pointed out, however, that to some extent deacetylation in the presence of alcohol by alkali (18) is a catalytic process and the amount of alkali required is not theoretically necessary for three acetyl groups.

It was then attempted to fix the position occupied by the potassium hydroxide molecule in the addition compound by the usual mild treatment with dimethyl sulphate. The solid remaining after such treatment was washed with acetone and thoroughly extracted with boiling methyl alcohol. This yielded a white residue (A) consisting of unchanged amylose, partially methylated amylose and inorganic salts. After removal of the potassium methyl sulphate from the methyl alcoholic

solution, precipitation with alcoholic potassium hydroxide, neutralisation and acetylation, a syrup (B) was obtained.

(A) was extracted with pyridine and the solution treated with acetic anhydride, on treatment a glass (A) was obtained having a methoxyl content of 2.4% and $[\alpha]_D^{15} +182^\circ$ ($c = 0.69$ in chloroform). The magnitude of the rotation was somewhat higher than that of amylose acetate, and lower than that of a methylated amylose (16), and the properties of the glass were consistent with the view that it was a specimen of a partially methylated amylose acetate and not a degradation product. The low methoxyl value however, makes it clear that the substance must be either (1) a mixture of a methylated amylose diacetate (theory OMe, 18.9%) (assuming no di- or tri-methyl amylose to be present) and amylose acetate or (2) a sample of amylose acetate which is substituted by methyl groups on about one in every six α -glucopyranose units (again assuming monosubstitution which appears likely since a rigorous search failed to reveal any polymethylated glucoses after hydrolysis). At present it is impossible to distinguish experimentally between possibilities (1) and (2).

In previous work on the subject of the structure

of the alkali hydroxide addition compounds of sugars (2) it has been repeatedly pointed out that the union between the hydroxyl groups and the alkali must be labile since the yields of methylated products obtained were invariably low. It is clear that even if all the glucopyranose groups of amylose carry one potassium hydroxyl residue, it by no means follows that a single treatment with methyl sulphate at 65-75°C. for 22 minutes will cause substitution of methoxyl for hydroxyl at every point of union between alkali and sugar-hydroxyl groups. Some will undoubtedly escape substitution just as potassium hydroxide-glucose fails to give a quantitative yield of methylglucoside. Another factor which may operate in this case is the tendency of amylose to aggregate and form more complex and insoluble structures with consequent loss of reactivity.

Haworth and his co-workers (22) have repeatedly pointed out that particularly in the case of starch there appears to be evidence of association between the chains of α -glucopyranose units, and that the phenomenon of the retrogradation of soluble amylose to an insoluble unreactive form similar to amylopectin is presumably due to some kind of association between

the chains, since the molecular weight determined by physicochemical methods is hugely increased, but the "chain length" remains constant at 24 glucose units. It is considered, therefore, that possibility (2) is the more probable.

(A') by deacetylation, hydrolysis, removal of glucose by precipitation with potassium hydroxide followed by acetylation yielded a syrupy monomethyl glucose tetra-acetate (yield 8%). From the corresponding free sugar on treatment with phenylhydrazine glucose phenylosazone was obtained. The yield of glucosazone from the tetra-acetyl monomethyl glucose amounted to 23% by weight. It is a well-known fact that 2-methyl glucose yields glucosazone in poor yield (24). A rigorous search failed to reveal the presence of any methylated osazones and it was therefore concluded that the methyl group was located at position "2" in the glucose molecule. This work was repeated three times with the same result in each case. It is considered improbable that the glucosazone on which this conclusion is based, was derived from glucose which had evaded removal and not from 2-methyl glucose.

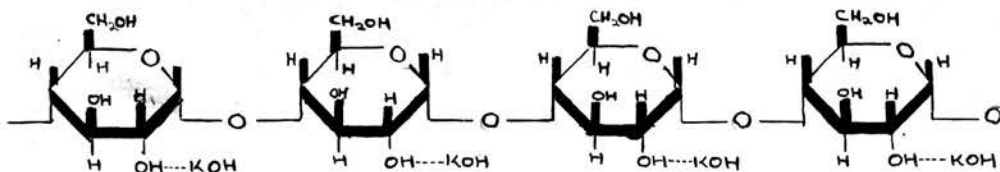
Examination of the syrup (B) (Yield 8%) revealed a methoxyl content of 12.4% which is in fair agreement

with the theoretical value for a diacetyl monomethyl amylose (11.9%). The specific rotation, however, $[\alpha]_{D}^{18} + 72.6^{\circ}$, indicated that such could not be the case, and B was clearly a degradation product, most probably a maltose derivative formed by hydrolysis of part of the amylose by the methyl sulphate. Although precautions were taken to ensure that the reagent was neutral, some local acidity may have developed during the reaction. The product was reducing to boiling Fehling's solution and a determination of the glucosidic methoxyl by a modification of the method of Freudenberg and Soff (19) (see p.145) indicated the absence of methoxyl in that position. Decetylation, hydrolysis and precipitation with potassium hydroxide followed by the addition of a large amount of ether to remove any free glucose which might have been present, followed by acetylation, yielded a syrup which appeared to be a monomethyl tetra-acetyl glucose. The addition of a large volume of ether removed some methylated compound since acetylation of the precipitate produced a product of OMe, 9%. This step was taken, although the yield of final product was thereby seriously impaired in order that no free glucose should escape precipitation.

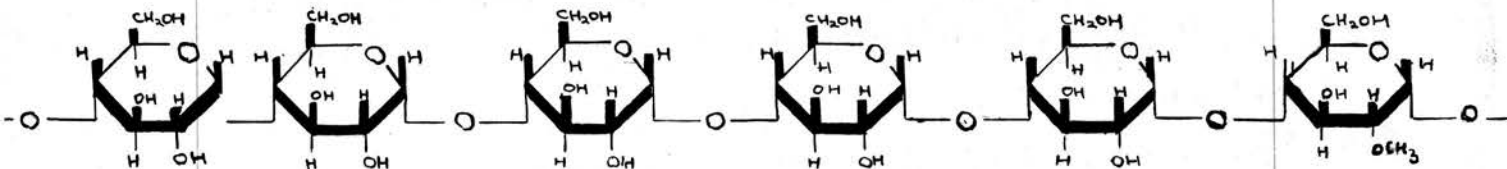
As before all attempts to isolate a monomethyl glucosazone failed, but from the syrupy acetate glucosazone in about 35% yield was obtained so that the presence of a methyl residue in position 2 of the monomethyl glucose may be presumed. This was several times confirmed. It was unfortunate however that no crystalline derivatives of 2-methyl glucose could be isolated to confirm this view. This result supports the view that the potassium hydroxide residue is associated with position 2 in the glucopyranose units, only if hydrolysis of the amylose took place after the substitution by methoxyl, since as shown in the preceding section maltose itself undergoes substitution in position 2 (23) (of the case of lactose (13)). The supposition that hydrolysis to maltose was the first step followed by migration of the potassium hydroxide from some other position to position 2 and subsequent methylation is, however, considered improbable.

Despite a careful search for other methylated glucose derivatives and in particular for 6-methyl glucose, none were discovered and in the absence of any evidence to the contrary it is considered that amylose combines with one molecular proportion of potassium hydroxide to form a compound in which the alkali residue

is associated with the hydroxyl group on the second carbon atom in the α -glucopyranose units. The formula for the alkali addition compound of amylose is essentially



and that reaction with methyl sulphate gives rise to an incompletely methylated monomethyl amylose such as



In order to establish early in the work whether the methoxyl found in the partially methylated derivatives was ethereal or glucosidic it was decided to employ a modification of Freudenberg and Soff's (19) hydrolytic method. It was concluded however that subject to the experimental error (2-3%) of the method, in no case did any methoxyl enter the reducing group.

EXPERIMENTAL.

THE PREPARATION OF AMYLOSE TRIACETATE.

It is found that attempts to prepare the acetylation product of starch by the usual methods generally fail or lead to inferior products and it is necessary to subject the starch to some preliminary activating treatment before acetylation. Of the three methods attempted that due to Baird, Haworth and Hirst (12) proved the most suitable.

(1) Method due to Baird, Haworth and Hirst. (12).

Air dried potato starch (50 gm.) was ground up in a mortar with absolute alcohol to a thick consistency and sufficient absolute ethyl alcoholic - hydrogen chloride then added to make the concentration of the acid 0.5%. (total volume 50 c.c.). The mixture was next refluxed for 30 minutes and the product filtered off and washed with absolute alcohol and finally with ether. This (45 gm.) was then added in small quantities to boiling water (500 c.c.) with constant stirring. When it had cooled somewhat alcohol was added (2500 c.c.) till no further precipitation occurred and after allowing time for the precipitate to settle out the supernatant liquid was decanted off and the solid triturated

123.

with cold alcohol, then washed with alcohol and ether and dried in a vacuum desiccator.

Yield 42 grams.

This last product was the material used in the first series of titrations for the determination of the alkali combining capacity of amylose. In appearance it was very similar to starch. It was found to be readily soluble in water immediately after preparation but in a few hours time had reverted to the insoluble form.

A rotation in water gave:-

$$\left[\alpha \right]_{D_s}^{160} + 180^{\circ} \quad (c = 0.2)$$

After drying for an hour in a vacuum the freshly prepared amylose (42 gm.) was shaken with a mixture of pyridine (130 c.c.) and water (20 c.c.) at room temperature for 18 hours. At the end of this period the amylose had swollen so much that the whole mass had become gelatinous. It was then subjected to acetylation with pyridine (300 c.c.) and acetic anhydride (300 c.c.) The acetylation mixture was heated on a water bath at 60°C. for 3½ days with constant stirring. The acetylated amylose was then poured into a large volume of cold water and the product precipitated as a fibrous mass. The

acetylated amylose was then washed with water for 24 hours when it was free of pyridine. On drying in vacuo a fine white powder was obtained.

Yield 45 grams.

Analysis:

Found: CH_3CO , 43.5%.

Calculated for amylose triacetate $\text{C}_{12}\text{H}_{16}\text{O}_8$

CH_3CO , 44.8%.

$[\alpha]_{\text{D}}^{16^\circ} + 171^\circ$ in chloroform ($c = 0.2$)

(2) Method due to Haworth, Hirst and Webb. (16)

In this method as before the starch was first subjected to a preliminary treatment and then acetylated. Ordinary air dried potato starch (20 gm.) was added to warm water (600 c.c.) in an enamelled container and stirred for 30 minutes. When the paste had cooled the starch was precipitated as a curdy mass by the addition of alcohol (1500 c.c.) The supernatant liquid was decanted off and the precipitate triturated with cold absolute alcohol. The prepared starch was then dried overnight in a vacuum desiccator.

Yield 18.5 grams.

A modification of Barnett's method (20) for the acetylation of cellulose was followed. To glacial acetic acid (180 c.c.) through which chlorine had been bubbled for one minute finely powdered freshly prepared amylose was added and the mixture stirred at room temperature. At the end of 30 minutes acetic anhydride (220 c.c.) containing an amount of sulphur dioxide equivalent to that of chlorine in the acetic acid was added and the stirring continued for a further 60 minutes. The acetylation was then completed by raising the temperature to 60°C. and the stirring continued for a further five hours. The acetylation mixture was then poured into a large volume of water and the precipitated product well washed with water. When acid-free it was filtered and dried in vacuo.

Yield 10 grams.

It was found difficult to estimate the requisite quantity of chlorine and the method was discarded in favour of that first described.

(3). Method due to Ling and Nanji. (21).

A third method for the preliminary treatment of starch for acetylation was attempted but this ~~was~~ was rejected owing to the unsatisfactory product obtained.

Amylose was separated from the amylopectin by heating a 5% aqueous starch paste (500 c.c.) for 30 minutes at 90-95°C. It was then cooled in a salt-ice freezing mixture and kept between -10° and -15°C. for a period of 6 hours. The more soluble amylose was leached out from the fibrous mass by means of warm water (60°C.). The aqueous extract was concentrated at 50°C. under reduced pressure and the amylose precipitated from the cold concentrate by addition of alcohol. The curdy precipitate was triturated as before with cold absolute alcohol and then filtered and washed and dried in vacuo.

Yield 4 grams.

The yield obtained was that quoted by Hirst, Plant and Wilkinson (22). An unsuccessful attempt was made to acetylate this product by means of chlorine and acetic anhydride (20).

METHOD EMPLOYED FOR THE PRELIMINARY INVESTIGATION
OF THE ALKALI COMBINING CAPACITY OF AMYLOSE.

It was mentioned in the introductory part of this section (p114) that the most satisfactory way of determining the combined alkali was by deacetylation of the acetate and compound formation at the same time by means of alcoholic potassium hydroxide in the medium of

alcohol. Two carefully weighed quantities of amylose triacetate (0.2024 gm. and 0.2014 gm.) of about the same weight were suspended in absolute alcohol and to each of the two suspensions was added a certain volume of standard alcoholic potash (10 c.c. of 0.946 N.) and the mixture allowed to stand $2\frac{1}{2}$ hours. Vol. alcohol = 2.22 c.c.

(1). The contents of the first tube were then analysed for the amount of alkali used up for deacetylation and compound formation together. The precipitated amylose potassium hydroxide compound was removed by filtration through a Gooch crucible and an aliquot part of the filtrate titrated against standard $N/10$ H_2SO_4 . The residue after washing with the minimum quantity of absolute alcohol was transferred to a flask containing water giving the "Direct" result.

(2). To the contents of the second tube water and indicator were added and titrated with standard $N/10$ H_2SO_4 and in this way the amount of alkali required for deacetylation was determined.

RESULTS:-

The normality of the alcoholic potassium hydroxide solution was 0.946 N.

The Initial Normality was 0.775 N and

. . Initial Weight of KOH present was 0.528 gm.

The Final Normality was 0.565 N and

. . Final Weight of KOH present was 0.386 gm.

(a) Weight of potassium hydroxide not used in deacetylation (titration (ii)) was 0.419 gm.

. . Weight of potassium hydroxide required for deacetylation was 0.528 - 0.419 gm. i.e. 0.109 gm.

. . 100 gm. amylose acetate will require for deacetylation 53.7 gm. potassium hydroxide.

(b) Now amount of alkali used up in deacetylation and compound formation was 0.528 - 0.386 gm. i.e.
0.142 gm.

. . Weight of potassium hydroxide used in compound formation (indirect) was 0.142 - 0.109 gm. i.e.
0.033 gm.

But weight of amylose corresponding to 0.2014 gm. amylose acetate is 0.1133 gm.

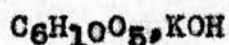
. . 100 gm. of amylose will react with $\frac{0.033 \times 100}{0.1133}$ gm

i.e. 29.1 gm. potassium hydroxide.

(c) From the direct titration of the solid compound we find that 100 gm. amylose will react with 30.5 gm. potassium hydroxide.

Similar experiments were conducted over a large range of alkali concentrations and the results are tabulated in Table No: 2. (p 110).

Theoretically for a compound



100 grams. of amylose will combine with 34.6 grams of potassium hydroxide.

A TYPICAL PREPARATION OF AMYLOSE - POTASSIUM HYDROXIDE.

Amylose acetate (40 gm.) was suspended in absolute alcohol (175 c.c.) and to this alcoholic potash (400 c.c.) of 2 N.) was added with stirring. The flask was then corked and set aside for three hours. Filtration of the addition complex and washing with the minimum quantities of absolute alcohol and dry ether to remove adhering potash solution was followed by drying in a vacuum desiccator over phosphoric anhydride overnight.

The product was a white, amorphous, slightly deliquescent powder.

Yield 30 grams.

Analysis:

The alkali content was determined by titration of a sample against N/10 H_2SO_4 . Found:

KOH 23.6%

calculated for 1:1 addition compound $C_6H_{10}O_5 \cdot KOH$.

KOH 25.7%

Or 100 gm amylose will combine with 34.6 gm. potassium hydroxide.

A TYPICAL METHYLATION OF AMYLOSE POTASSIUM HYDROXIDE.

To the finely powdered amylose-potassium hydroxide compound (25 gm.) contained in a flask fitted with a stirrer, dimethyl sulphate (150 c.c.) previously neutralised with anhydrous potassium carbonate was added. The flask was surrounded by a water bath which was maintained at $65^{\circ}C$. for 15 minutes and then quickly raised to $75^{\circ}C$. at which temperature it was kept for $7\frac{1}{2}$ minutes. The contents were vigorously stirred all the time. Towards the end of the second period of heating it was noticed that the reaction mixture had become slightly viscous and was seen to stick to the walls of the flask. After cooling in ice the supernatant liquid was decanted off and the residue thoroughly washed with acetone to free it from dimethyl sulphate. This was followed by extraction

of the methylated syrup with hot dry methyl alcohol and the extract was then set aside for a few hours to allow the potassium methyl sulphate to crystallise out. The white powdery residual material which was inextractible with hot alcohol was then examined. The examination of the material soluble in alcohol is reported later on p.138.

EXAMINATION OF THE RESIDUAL MATERIAL INEXTRACTIBLE
BY METHYL ALCOHOL AFTER METHYLATION.

As pointed out in the discussion to this section it was considered that the portion of the methylation product soluble in methyl alcohol was to some extent a degradation product and that the white residual material probably consisted of unchanged amylose, partially methylated amylose and inorganic salts. It was thus decided to examine this product first owing to its undegraded nature.

After washing with acetone several times to remove any remaining dimethyl sulphate the residual substance (15 gm.) was finely powdered in a mortar and then thoroughly extracted with pyridine (300 c.c.) at 65°C. To this, acetic anhydride (150 c.c.) was added and the

whole kept at 68-70°C. for three hours. After standing for two days at room temperature the reaction mixture was poured into cold water and the acetylated material extracted several times with chloroform (500 c.c.). The pyridine in the extract was removed by washing with dilute sulphuric acid and then after treatment with solid sodium bicarbonate the chloroform solution was dried over anhydrous sodium sulphate and taken to dryness in the usual way. The resulting product was a yellow friable glass.

Yield 4.2 grams.

Analysis:-

Found: OMe, 2.44%, CH₃CO, 40.3%

Freudenberg and Seff glucosidic methoxyl determination

Found: OMe, 1.72%

"experimental" error 2 - 3% OMe.

$[\alpha]_{\lambda = D.}^{16^{\circ}C.} + 182^{\circ}$ in chloroform (c = 0.69).

HYDROLYSIS OF THE PARTIALLY METHYLATED
AMYLOSE ACETATE.

The acetylated product was combined with another identical glass obtained at the same stage in a previous experiment and the combined mass deacetylated by the method of Zemplen (17). To the cold chloroform solution (50 c.c.) of the syrups (12.5 gm.) a cold solution of sodium (1.0 gm.) in dry methyl alcohol (40 c.c.) was added and the whole immersed in a basin of cold water. At the end of three hours sufficient acetic acid was added to neutralise the sodium and the deacetylated syrup was extracted twice with water (65 c.c.) as before.

The requisite quantity of sulphuric acid was added to the aqueous extract to make it 1.5 N. and this was heated on a water bath at 95°C. till constant rotation was attained. The solution was neutralised with barium carbonate and, after the removal of the insoluble barium salts and thorough leaching of the mass with warm water, was evaporated to dryness at 45-50°C. under reduced pressure to give a syrup somewhat contaminated with inorganic material. The hydrolysed syrup was then extracted by several

treatments with warm absolute alcohol and to the cold extracts excess of alcoholic potash (15 c.c. of 2 N.) was added. The precipitate (I) obtained was filtered off and to the filtrate dry ether (1250 c.c.) was added till no further precipitation (II) occurred. These precipitates were washed with small quantities of alcohol and ether to remove any adhering potash solution and dried in a vacuum desiccator. The filtrate was acidified and taken to dryness.

EXAMINATION OF THE PRECIPITATES.

The dried sugar alkali compounds were hygroscopic powders readily soluble in water.

Precipitate	(I)	(II)
Yield	6.0 gm.	3.5 gm.

A small quantity (1 gm.) of each was dissolved in water, acidified with glacial acetic acid and treated with pure phenylhydrazine (0.5 c.c.) and sodium bisulphite. After heating at 95°C. for about twenty minutes precipitates appeared. By recrystallisation from aqueous alcohol fairly pure specimens of the osazones were obtained.

Osazone	(I)	(II)
Yield	0.13 gm.	0.09 gm.
m.p.	198°C.	197°C.
OMe - %	nil	nil

The melting points of the specimens were not depressed on admixture with a known sample of glucosazone.

Second portions of the addition compounds were dissolved (separately) in water and neutralised carefully with dilute acetic acid. After taking to dryness under reduced pressure, acetylation and subsequent deacetylation and osazone formation confirmed the results above in both cases. It is to be noted, however, that the acetylated product from (II) contained a small amount of methoxyl, estimated at 1%. This would correspond to the presence in the acetate of 0.9 gm. of monomethylhexose tetra-acetate. The addition of the excess of ether had ~~indirectly~~ precipitated a quantity of the monomethyl glucose along with the alkali addition compound, but since it was necessary to remove all the free glucose this could not be avoided.

ACETYLATION OF THE FILTRATE.

The syrup obtained after the acidification of the ethereal filtrate with glacial acetic acid and evaporation to dryness was nearly colourless. It reduced Fehling's solution on boiling. By means of several treatments with hot pyridine (70 c.c.) the syrup was extracted and to the extracts acetic anhydride (35 c.c.) was added and the whole heated on a water bath at 65°C. for three hours. After standing at room temperature for two days the acetylation mixture was poured into a large volume of water and thoroughly extracted with chloroform (350 c.c.). The pyridine was removed by washing with dilute sulphuric acid and the excess acid by the addition of solid sodium bicarbonate. The chloroform extract was then dried over anhydrous sodium sulphate and evaporated to dryness at 45-50°C. under diminished pressure yielding a viscid syrup.

Yield 0.31 grams.

Analysis:-

Found: OMe, 8.65%

calculated for a monomethyl tetra-acetyl glucose

C H O .
15 22 10

OMe, 8.57%

$[\alpha]_{\lambda = D}^{16^\circ C.} + 32.30$ in chloroform (c = 0.31)

DEACETYLATION AND OSAZONE FORMATION.

The acetate syrup (0.3 gm.) was dissolved in dry chloroform (10 c.c.) and when well cooled was added to a similarly cooled anhydrous methyl alcoholic solution (10 c.c.) of sodium (0.01 gm.) and allowed to stand in a basin of cold water with occasional agitation for a period of three hours. The theoretical quantity of acetic acid for neutralisation was added and the de-acetylated syrup extracted with water (10 c.c.). A further aqueous extract (5 c.c.) was added to the first and the combined extracts treated with chloroform (8 c.c.). To this aqueous solution phenylhydrazine (0.3 c.c.), sodium acetate (0.2 gm.) and a few drops of glacial acetic acid were added and the mixture heated on a water bath at 95°C. Within a short time (45 minutes) an osazone had formed which was filtered off. In a like manner a second crop was obtained.

Yield 0.07 gms.

Analysis:-

The crude osazone was recrystallised once from aqueous pyridine and it was found that the crystalline form was identical with that of glucosazone.

m.p.	196°C.
OMe-%	nil

mixed melting point with an authentic specimen of glucosazone showed no depression. The absence of methoxyl in the osazone showed that the methoxyl group must have been attached to the second carbon atom in the glucose molecule.

EXAMINATION OF THE MATERIAL EXTRACTED
BY HOT METHYL ALCOHOL.

The hot methyl alcohol soluble fraction was now examined.

To the potassium methyl sulphate-free alcoholic solution an excess of alcoholic potash (100 c.c. of 2 N.) was added and to complete the precipitation a large quantity of dry ether (2000 c.c.) was poured in. These precipitates were filtered off at the pump, washed with small quantities of absolute alcohol and dry ether and dried in a vacuum desiccator.

Precipitate	First	Second
Yield	3.35 gm.	1.84 gm.

In this manner the unmethylated compounds e.g. maltose and degraded amyloses, were removed in the form of the

alkali addition compounds.

The residual solution was acidified with glacial acetic acid and, when the precipitated potassium acetate was removed, was taken to dryness on a water bath at 40°C. under diminished pressure.

ACETYLATION OF THE METHYLATED
SYRUP.

The dry mass of syrup and potassium acetate was extracted several times with warm pyridine (100 c.c.). Acetic anhydride (50 c.c.) was added and the acetylation kept at 65°C. for 2½ hours. It was then allowed to stand at room temperature for two days. The acetylation mixture was poured into a large volume of water, extracted thoroughly with chloroform, washed well with dilute sulphuric acid and the excess acid neutralised with solid sodium bicarbonate. After drying in the usual way over anhydrous sodium sulphate the chloroform extract was evaporated to dryness at 45-50°C. on a water bath and under reduced pressure. The resulting product was a clear light brown coloured syrup.

Yield 1.52 grams.

Analysis:-

Found: OMe, 12.4%, CH₃CO 37.7%

calculated for a diacetyl monomethyl amylose $C_{11}H_{16}O_7$

OMe, 11.9%, $\frac{CH_3CO}{3}$, 33.1%

calculated for a dimethyl hexa-acetyl maltose $C_{26}H_{36}O_{17}$

OMe, 9.98%, $\frac{CH_3CO}{3}$, 41.5%

A Freudenberg and Soff glucosidic methoxyl determination gave

OMe, 2.44%

"Experimental" error 2 - 3% OMe.

$[\alpha]_D^{18^\circ C.} + 72.6^\circ$ (c = 0.52) in chloroform.

HYDROLYSIS OF THE SYRUP.

Deacetylation followed employing Zemplén's (17) catalytic sodium methoxide method. The syrup (1.3 gm.) was dissolved in dry chloroform (10 c.c.) and when ice cold was added to a similarly cooled dry methyl alcoholic solution (10 c.c.) of sodium (0.4 gm.). The mixture, surrounded by cold water, was allowed to stand three hours. At the end of this period a slight excess of acetic acid was added and the deacetylated sugar

extracted with water (10 c.c.), followed by further treatment of the chloroform extract with water (10 c.c.) and of the aqueous extracts with chloroform (10 c.c.). The aqueous extracts were combined (25 c.c.) and hydrolysed. Sufficient dilute sulphuric acid was added to make the resulting solution 1.5 N. This was heated on a water bath at 95°C. till constant rotation was attained - the period required was 6 hours. The acid solution was next neutralised with barium carbonate and after thorough washing of the residue with hot water to remove all the hydrolysed syrup the extract was evaporated down to dryness at 45-50°C. under diminished pressure. The last traces of moisture were removed with the aid of a benzene-alcohol mixture. The syrup was next extracted several times from the inorganic impurities with warm absolute alcohol and, when cold, excess of alcoholic potash (30 c.c. of 2 N.) was added and then anhydrous ether (3000 c.c.) till no further precipitation took place. This residue was examined and the filtrate acetylated.

EXAMINATION OF THE PRECIPITATE.

The precipitated compound when dried was very hygroscopic.

Yield 0.61 grams.

An osazone was prepared from this product by treatment with phenylhydrazine and acetic acid in the usual way.

Yield 0.14 grams.

Analysis:-

After recrystallisation from aqueous alcohol.

OMe	nil
m.p.	200°C.
m.m.p.	200°C. with glucosazone).

The same precipitated product (0.50 gm.) from a later experiment was dissolved in water and carefully neutralised with dilute acetic acid and taken to dryness. Acetylation with pyridine (25 c.c.) and acetic anhydride (12.5 c.c.) gave, after the usual working up, a small quantity of a dark syrup devoid of methoxyl.

From these two facts the precipitated complex was concluded to be the alkali addition compound of glucose.

In this way an approximate fractionation of the mixture was achieved.

ACETYLATION OF THE FILTRATE.

The ethereal filtrate, after the precipitation of the glucose, was acidified with glacial acetic acid and the precipitated potassium acetate filtered off and the liquid taken to dryness on a water bath at 45°C . under reduced pressure. The resulting syrup was slightly yellowish in colour and showed reducing properties towards Fehling's solution. Acetylation followed. The syrup was extracted several times with hot pyridine (65°C .) and to the combined extracts (40 c.c.), acetic anhydride (20 c.c.) was added and the mixture kept at 65°C . for three hours. After standing at room temperature for 48 hours the acetylation mixture was poured into a large volume of water and extracted with chloroform (300 c.c.). This extract was then washed with dilute sulphuric acid until free of pyridine, and the excess acid neutralised with solid sodium bicarbonate.

After drying over anhydrous sodium sulphate, the

extract was evaporated down to dryness under reduced pressure. The resulting acetylated product was a yellow syrup.

Yield 0.15 grams.

Analysis:-

Found: OMe, 8.37%.

calculated for a monomethyl tetra-acetyl glucose

C H O
15 22 10.

OMe, 8.57%

$\left[\alpha \right]_{\lambda = D}^{14^{\circ}C.} + 66.7^{\circ}$ in chloroform (c = 0.66)

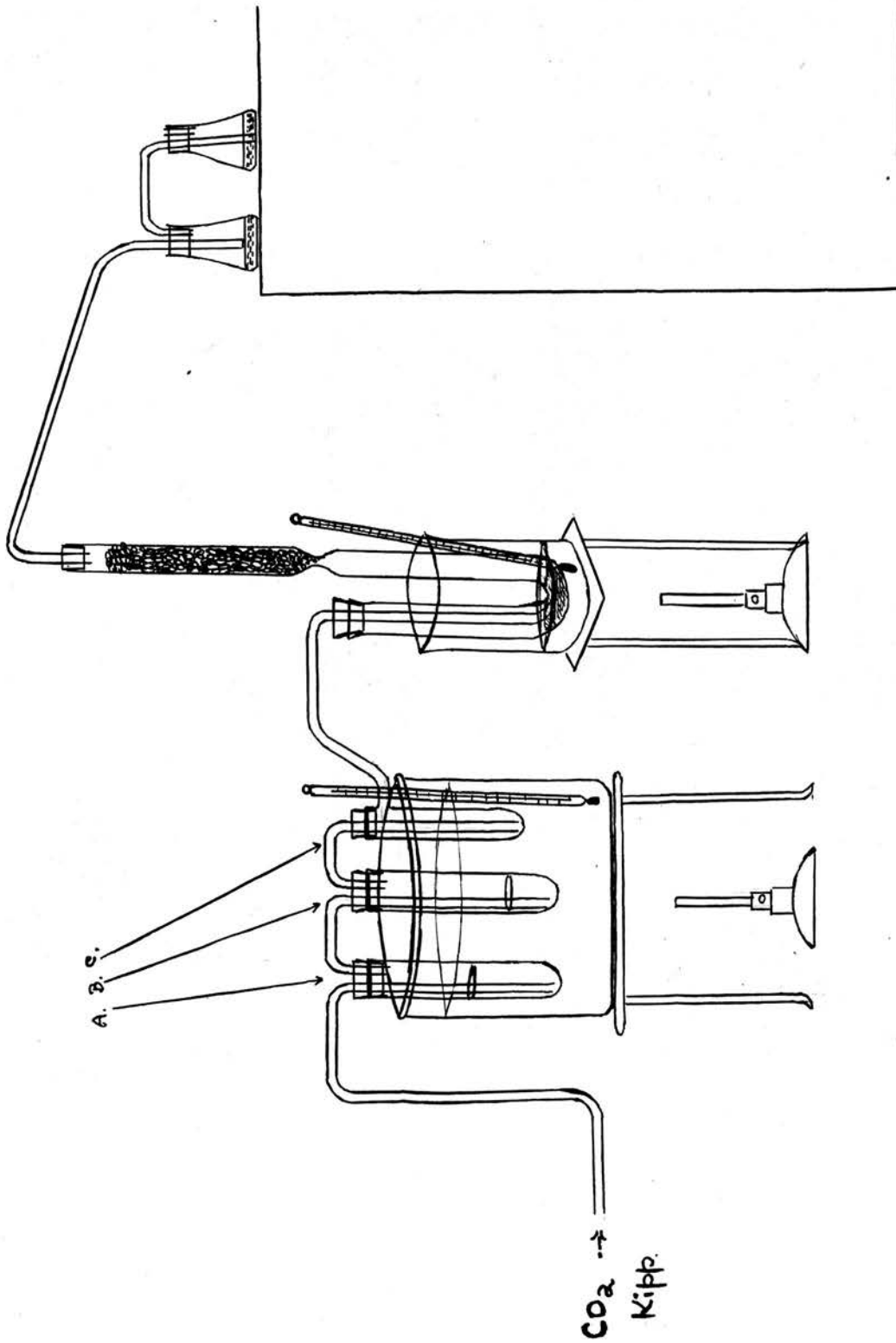
DEACETYLATION AND OSAZONE FORMATION.

The acetate (0.1 gm.) was now deacetylated by Zemplén's (17) method. To the cold chloroform solution (10 c.c.) of the syrup a cold methyl alcoholic solution (10 c.c.) of sodium (0.02 gm.) was added and after standing in cold water for a period of three hours the deacetylated syrup was extracted with water in the

glucosidic or ethereal.

In this a weighed quantity of the substance to be examined was hydrolysed with dilute sulphuric acid for a definite period ($1\frac{1}{2}$ hours) and the methyl alcohol produced was estimated in an ordinary Zeisel apparatus. Since the reducing group is the only one to be attacked it forms a ready and easily applied method for estimating this group. From the results, however, it appeared that a small quantity, between 2% and 3%, of methoxyl was present, but on running a test with a known sample of trimethyl starch and later with tetramethyl-glucose it was found that there was an experimental error in the method amounting to the percentage quoted. According to Freudenberg and Soff's paper blanks were made on several sugars and in the case of tetramethyl-glucose no precipitate was obtained. In all the cases to which this method was applied a 2-3% glucosidic methoxyl content was obtained but as this was written down to experimental error it was assumed that no methoxyl was present in the glucosidic position.

Experimentally the apparatus (diagram p 147) consisted of three wide-necked boiling tubes each fitted with two-holed rubber stoppers. The boiling tubes are connected with each other by means of narrow bore glass

DIAGRAM - I

tubing the inlet tube in each case being cut off a few mms. short of the bottom of the boiling tube. The outlet tube from the last container "c" was led right into the hydriodic acid in the bottom of the Zeisel apparatus. The gaseous products were trapped in the usual way in saturated alcoholic silver nitrate. 7% H_2SO_4 was employed as the hydrolysing agent.

Tube "a" - contained 50 c.c. of 7% H_2SO_4 .

Tube "b" - contained 10 c.c of 7% H_2SO_4 and a weighed quantity of the substance to be analysed.

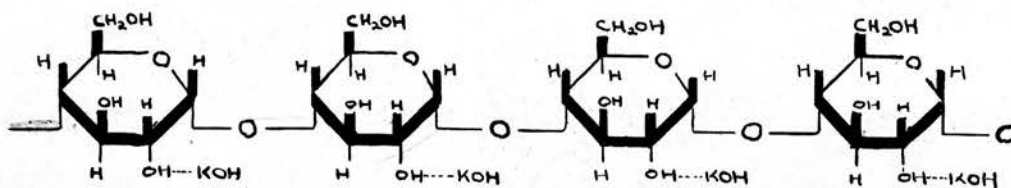
Tube "c" - was a trap to avoid dilution of the hydriodic acid.

Carbon dioxide was bubbled through the apparatus at the rate of one bubble per second. In this way any methyl alcohol formed was forced into the hydriodic acid and converted to methyl iodide which was then trapped in the silver nitrate. The hydrolysis mixture was kept at $100^{\circ}C$. during the running of the experiment by means of a boiling water bath.

S U M M A R Y.

- (1). Titration experiments with amylose acetate showed that amylose would combine with one molecular proportion of potassium hydroxide. The weaker concentrations of alkali favouring the better product.
- (2). Mild methylation of this product gave rise to two fractions, a portion soluble in methyl alcohol and which was considered to consist mainly of degraded amylose and an insoluble portion consisting of normal amylose.
- (3). Acetylation, deacetylation and hydrolysis then reacetylation of the insoluble portion gave the acetate of a monomethyl glucose which by further treatment was shown to be 2-methyl glucose.
- (4). Treatment in the same way of the methyl alcohol soluble portion gave similar results.
- (5). From a consideration of all the facts the formula

of the alkali addition compound of amylose was found to be such that potassium hydroxide residues are attached to the hydroxyl groups of carbon atom 2 in the glucopyranose units.



B I B L I O G R A P H Y.

- (1) Haworth "Constitution of Sugars"
(Arnold & Co.) 1929.
- (2) Percival J.C.S., 1934, 1160.
- (3) Nageli "Die Starkekörner" - 1858.
- (4) Schardinger Centr. B. Par., 1908, 22, 98.
- (5) Pringsheim Ber., 1922, 55, 1444.
- (6) Karrer Helv., 1921, 4, 169.
- (7) Samec Kolloidchem Beihefte,
1916, 8, 33.
- (8) Liepatov Kolloid. Zeit., 1926, 39,
9.
- (9) Karrer Helv., 1921, 4, 811.
- (10) Karrer, Stueb and Walti... Helv., 1922, 5, 129.
- (11) Karrer, Nageli and
Hurwitz Helv., 1921, 4, 678.
- (12) Baird, Haworth and
Hirst J.C.S., 1935, 120.
- (13) Percival and Ritchie J.C.S., 1936, 1765.
- (14) Peiser (E.) Z. physiol. chem., 1926,
161, 210.
- (15) Bergmann and Knehe Ann., 1927, 452, 141.
- (16) Haworth, Hirst and Webb .. J.C.S., 1928, 2681.
- (17) Zemplén and Kunz Ber., 1923, 56, 1705.
- (18) Hamilton J.A.C.S., 1934, 56, 487.
- (19) Freudenberg and Soff Ann., 1932, 494, 68.

- (20) BarnettJ.S.C.I., 1921, 40, 8T.
- (21) Ling and NanjiJ.C.S., 1923, 123, 2666.
- (22) Hirst, Plant and
 WilkinsonJ.C.S., 1932, 2375.
- (23) HeddleThis Thesis p. 64.
- (24) Haworth, Hirst and
 Teece1931, 2858.

PART V.

**THE ADDITION COMPOUNDS OF CELLULOSE
AND POTASSIUM HYDROXIDE.**

DISCUSSION.

The nature of the solid phase when cellulose is brought into contact with solutions of sodium hydroxide and to a lesser degree potassium hydroxide of varying concentrations has been the subject of numerous researches over a period of nearly ninety years.

It was Mercer (Brit. patent No:13296/1850) who, in 1844, noticed that cotton when immersed in concentrated soda lye, became transparent and that considerable swelling and shrinkage of the fibres occurred. He also found that the soda could be readily washed out with water and that the fibres then again became opaque and exhibited a markedly increased chemical reactivity. All the changes produced by mercerisation have proved of great technical importance, notable amongst which are the "creping" effect, increased tensile strength, greater dyestuff absorption, silk-like lustre etc., This mercerisation effect of cotton was later shown to have been known in France at the time when Mercer made his discovery (1). That the combination of the soda with the cotton has effected a permanent change upon it is certain but the question arises as to the nature of the change.

In contrast to the other alkali addition compounds studied the literature on alkali cellulose is so voluminous that a comprehensive study of it is not easy. The published data have aimed to show that cellulose and caustic react to form a chemical compound or that the combination of the two is merely a physical one and in the case of the former whether additive or substitutive.

According to the earliest investigators the mercerization process was to be explained as the result of chemical combination between cellulose and sodium hydroxide followed by decomposition of the resulting compound with water. From this it was assumed that the compound could be compared with sodium ethoxide and other alcoholates.

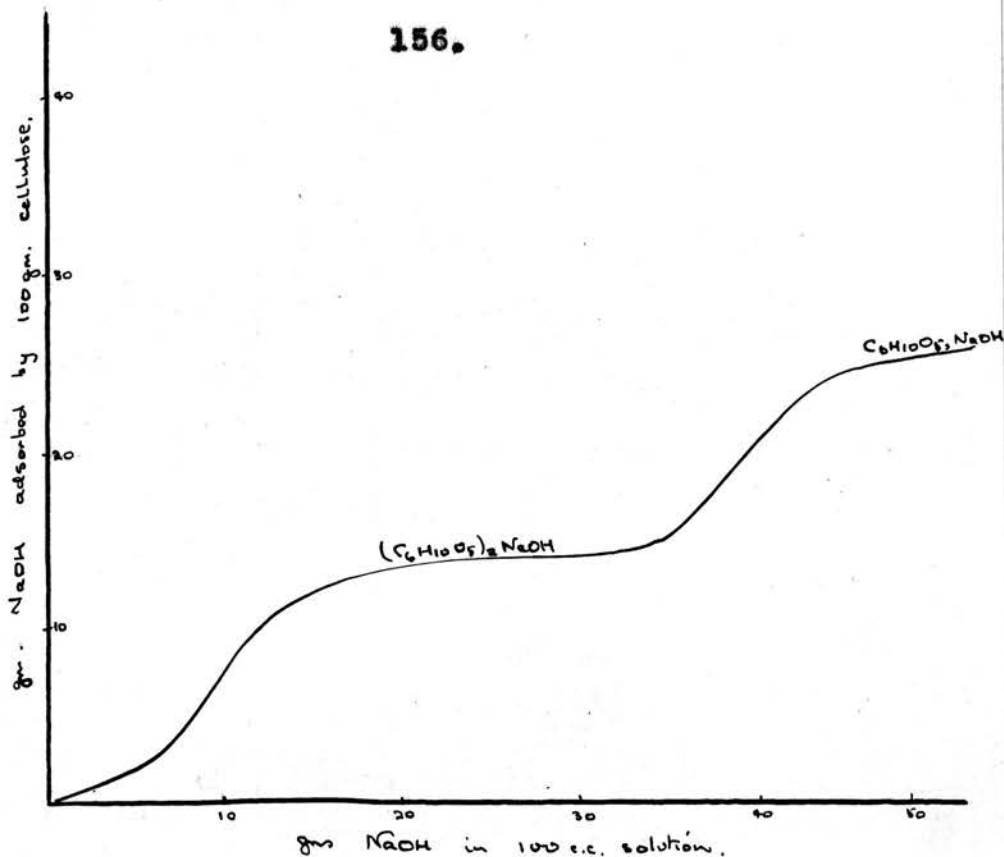
Mercer believed that such a chemical compound was formed at a certain optimum concentration and this idea has received considerable experimental support from the work of Gladstone (2), Thiele (3), Vieweg (4), Normann (5) and more recently by Percival, Guthbertson and Hibbert (6) and Rassew and Wolf (7).

Gladstone (2), who immersed cotton in soda solutions of various concentrations and after expressing the liquor and repeated washings of the product with absolute alcohol and determination of the alkali content gravi-

metrically, suggested that in mercerisation a definite chemical compound "soda cellulose" $(C_6H_{10}O_5)_2NaOH$ was formed. Hübner and Teltscher (33) cast some doubt on Gladstone's work and were unable to repeat his results. They showed that repeated washing with alcohol had a definite effect on the alkali content of the product.

Vieweg (4) determined the change in concentration between the original and final mercerisation liquors, the difference indicating the amount of soda taken up by the cotton, was able to show graphically that compound formation occurred. The results indicated the existence of $(C_6H_{10}O_5)_2NaOH$ and the probability of $C_6H_{10}O_5$, $NaOH$ (diagram I.). The experimental evidence was later repeated by Vieweg (8) with little change, owing to contrary statements by Rassow and Wadewitz (9) whose conclusions he stated were found to be based on analytical errors.

Heuser and Niethammer (10) were of the opinion that Gladstone's direct method of washing with alcohol was only applicable under certain conditions due to the tendency of the alcohol to concentrate the alkali on the fibre and they developed an extraction method. In this manner they were able to confirm Vieweg's absorption curve.



Karrer (11) similarly found evidence for the existence of $(C_6H_{10}O_5)_2NaOH$ and drew attention to the analogous compounds of starch, inulin and the various polymerides of anhydromaltose classed as amyloses.

Katz (12 and 20) whose preliminary work agreed with Vieweg's, found that by using aqueous alcoholic solutions the flat portion of the absorption curve was absent and that a linear relationship was obtained, which was thought to indicate the formation of solid solutions of sodium hydroxide in cellulose. By examining both cases spectrographically it was found that an alkali cellulose compound was formed in the aqueous alcoholic solutions since the observed bands were similar to those obtained using aqueous solutions

and it was thought that the presence of alcohol hindered the formation of the compound.

The action of strong bases on cotton cellulose was investigated by Dehnert and König (13,14) and they gave evidence for the existence of both $(C_6H_{10}O_5)_2NaOH$ and $(C_6H_{10}O_5)_2NaOH$. Potassium hydroxide was found to form a complex $(C_6H_{10}O_5)_2KOH$ but there was no evidence of any further combination. D'Ans and Jaeger (15) also confirmed Vieweg's second compound at high alkali concentrations whilst Karrer (11) and Hess (16,17) deny this.

Knecht and Platt (18) indicated the formation of a compound $(C_6H_{10}O_5)_2NaOH$ by measurement of the preferential absorption of sodium hydroxide by cotton yarn. They obtained similar measurements, although less conclusive, supporting the formation of a compound $(C_6H_{10}O_5)_2KOH$. Liepatoff (19) found that the curve for potassium hydroxide had the same general character as that for sodium hydroxide.

It must be pointed out, however, that Vieweg's interpretation of his curve is theoretically impossible. It is, of course, customary to determine the amount of adsorption from the change of concentration but this method rests on the assumption that no

water is taken up by the solid phase and it becomes inaccurate the moment this assumption fails to hold. Percival, Cuthbertson and Hibbert (6) showed that the amount of water adsorbed by the cellulose during the alkali treatment reached a maximum with 14% sodium hydroxide. Further, in Vieweg's curve, if compounds exist along the ordinates then there must be two phases along the other curves; the concentrations of these solutions should remain constant and these curves should be parallel to the abscissa. It is a case of a three component system cellulose-sodium hydroxide-water which, of course, cannot be dealt with two dimensionally. Thus Vieweg's hypothesis does not agree with his own data. In other words the horizontal parts of the curve do not necessarily indicate stoichiometric combinations.

Percival, Cuthbertson and Hibbert (6) studied the absorption of sodium hydroxide by cellulose over a wide range of concentrations and by a carefully controlled method of washing the product they were able, by means of extrapolation, to give evidence for the formation of a compound between alkali concentrations of 12.5% and 21.5% by weight. The compound was regarded as resulting from the interaction of one molecule of sodium hydroxide with each anhydroglucose unit of the

cellulose. Quantitative analysis of isolated samples of alkali cellulose indicated a mixture of the forms $(C_6H_9O_5Na)_x$ and $(C_6H_{10}O_5, NaOH)_x$ and the suggestion was made that the product may be the result of an equilibrium action of the nature $(C_6H_{10}O_5, NaOH) \rightleftharpoons (C_6H_9O_5Na)_x \cdot xH_2O$.

Rassow and Wolf (7) on the other hand, by means of washing the mercerised cotton with cold absolute alcohol to absolute neutrality employing alizarin-yellow as indicator (21) claimed to have isolated a definite compound of the formula $C_{12}H_{20}O_{10} \cdot NaOH$. By means of a series of accurate analyses they were able to show that the product, soda-cellulose, was probably additive, i.e. not an alkoxide.

On the other hand the combination of cellulose and sodium hydroxide is viewed by some from a colloidal chemical standpoint as an adsorption phenomenon.

Leighton (22) observed that the contradictory conclusions of the many workers in this field could be traced to the unsatisfactory processes of removing the adhered alkali from the mercerised cotton and claimed that by centrifuging the superfluous liquor could be

removed. His results showed that the amount of sodium hydroxide taken up by the cellulose was a continuous function of the concentration of the solution and there was no evidence to justify the assumption of formation of a definite chemical compound. Coward and Spencer (23) in a similar manner concluded that the phenomenon was due to adsorption. Whilst Millar (24,24,26, and 27) was of the opinion that the distribution coefficient ratio of alkali in the solid phase to alkali in the liquid phase indicated a case of solid solutions and Liepatoff (19) considered the absorption of sodium hydroxide by cellulose as purely chemical absorption.

Nevertheless the weight of evidence from other sources, as well as that obtained by most investigators using the indirect method of estimating the combined alkali tends to strengthen the belief that a chemical compound is formed and this belief is further supported from the results of physico-chemical work.

The conception of the formation of a chemical compound of cellulose with alkali has been somewhat verified by X-ray analysis. With the hydroxides

of lithium, potassium and sodium the X-ray point found to coincide with the break in Vieweg's curve (30). This fact, in conjunction with that discovered by Heuser (28) that the quantity of alkali absorbed in the flat portions of the curve was equimolecular for the three alkalis, indicated chemical combination and not adsorption. Clark (29) with mercerised cellulose showed that a double line appeared in the X-ray pattern which supported the view that mercerisation was a definite chemical process. Herzog (31) showed that the absorption bands in the infra red region of the spectrum for cellulose and mercerised cellulose were distinctly different.

The mechanism of the reaction between cellulose and caustic soda cannot be explained satisfactorily till the question of the nature of the product "soda-cellulose", is finally settled and if a definite chemical compound its structure investigated. Some workers have shown the reaction to be ionic. Heuser and Bartunek (32), in particular, showed that the degree of swelling of the cellulose fibres in lithium, potassium, sodium, rubidium and caesium hydroxides proceeded with increase in concentration of alkali up to the point corresponding to the formation of the chemical compound in each case

$(C_6H_{10}O_5)_2XOH$, since they could find no evidence for $C_6H_{10}O_5, XOH$. This maximum of swelling occurred at the

same concentrations as the maximum electrical conductivities of their solutions and was dependent on the degree of hydration of the alkali ions in the bases used. The lithium ion, which is the most hydrated, caused the greatest swelling whilst the caesium ion, the least hydrated, caused the least volume change in the cellulose. The fact, that alkali cellulose is not formed in alcoholic solution lends support to the ionic nature of the reaction.

Very little has been done on the treatment of cellulose with potash solutions except that incidental to the soda work. It was found in this present work, however, that the potash cellulose product was much more susceptible to methylation under the mild conditions employed than the soda compound and for the sake of uniformity it was decided to investigate the case of the potash compounds. It is very improbable that there is any essential difference between the two cases.

The treatment of the cotton was carried out as recommended by Percival, Cuthbertson and Hibbert (6) employing a 35% aqueous solution of potassium hydroxide in place of the sodium hydroxide. The time of immersion was four hours, a period which in the experience of other workers (24,25,25,27) would be ample for equilibrium to be established. The wet fibre was then dried for three

minutes using suction and subjected to a single standardised washing with absolute alcohol as it had been shown that practically all the adhering alkali could be removed without affecting the absorbed alkali in this way. After drying over phosphorus pentoxide the addition product was analysed. The average alkali content in seven separate experiments was 24.3% KOH. For a compound $(C_6H_{10}O_5, KOH)_x$, KOH = 25.7%.

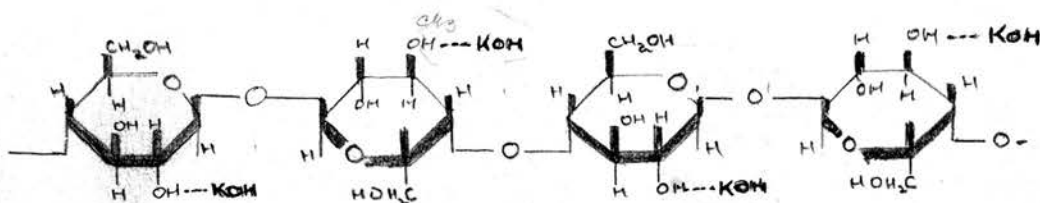
The position occupied by the potassium hydroxide residue in the addition compound was fixed by the usual mild treatment with neutral dimethyl sulphate. The product obtained seemed to vary greatly in methoxyl content (3 - 12%), the least change in conditions making a considerable difference in the analytical results. An attempt was made to separate out the methylated cellulose and use was made of Schweitzer's reagent for this purpose. It was found that although the product after treatment had a considerably enhanced methoxyl content yet on further treatment the methylated cellulose itself was dissolved and in order to raise the methoxyl to that corresponding to a monomethyl cellulose a considerable amount of the product had to be sacrificed. Owing to the poor yields, however, it was decided to abandon the attempt at separation in favour of direct hydrolysis of

the partially methylated cellulose. The method employed was that due to Haworth and Machemer in which the methylated product is dissolved in concentrated hydrochloric acid and then subjecting the solution to periodic saturation with hydrochloric acid gas at -20°C . After neutralisation of the acid most of the glucose was removed by fermentation with yeast and the residual sugar, after being converted to the glucoside was acetylated and fractionated. In this way three fractions were obtained.

- (1). A small quantity of a dimethyl diacetyl methylglucoside which was not further examined.
- (2). The second fraction consisted of a monomethyl triacetyl methyl glucoside which was shown to be derived from 2-methyl glucose by the isolation of glucosazone from the deacetylated and hydrolysed syrup. When a portion of this syrup was acetylated again the methoxyl content was found to be 9.94%. corresponding to a monomethyl glucose acetate.
- (3). The third fraction consisted of methylglucoside derived from glucose which had escaped removal during fermentation. This was confirmed by the ready formation of glucosazone from the deacetylated and hydrolysed syrup.

From the foregoing facts, therefore, the deduction

is made that one molecular proportion of potassium hydroxide combines with one molecular proportion of cellulose to form a compound in which the alkali residue is closely connected with the hydroxyl grouping on the second carbon atom in each β -glucopyranose unit making up the cellulose molecule.



The isolation of a dimethyl fraction in small quantity (2%) shows that there may also exist a di-alkali cellulose compound but further work will be required to elucidate the structure of this second complex. It is possible, however, that this may be a 2:6 derivative since it has been shown that in both β -methylglucoside and cellibiose (glucose β -glucoside) position 6 appears to be the most reactive towards alkali.

Piwonka (35) who methylated a Normann cupricellulose product with methyl sulphate found that on hydrolysis of the product a methyl methylglucoside was obtained which yielded a methyl glucosazone showing that position 2 was not substituted. The melting point of this product was found to be 178-180°C. corresponding to

3-methyl glucosazone, yield 52%. The yield of glucosazone obtained under identical conditions was 54%. Oxidation of the methyl methylglucoside with nitric acid and esterification of the product gave a monomethyl saccharo diethyl ester which appears to exclude substitution in positions 4 and 6. This author claims substitution to take place exclusively in position 3. No yields of these oxidation products are recorded however although Traube, Piwonka and Funk (44) recorded a number of experiments on the methylation of thallium cupricellulose and describe a number of partially methylated celluloses amongst which occurs a "hemimethyl" cellulose (OMe, 8.2.). From the hydrolysis products of these complex mixtures it is concluded that in only about 1.6% of the glucose groups is more than one hydroxyl group substituted by methoxyl whereas 55.0% contain one methoxyl group and 40.4% are substituted. No experimental evidence is presented however as to the whereabouts of the methoxyl residue in the monomethyl glucose.

Lieser and his co-workers (39,40,41,42,43.) have contributed many papers in an attempt to elucidate the structure of cellulose xanthates. He showed that by methylation of cellulose with nitroso methylurethane

in methyl alcohol a monomethyl dicellulose insoluble in organic solvents was obtained. On hydrolysis with 75% H_2SO_4 and subsequent fermentation of the glucose a monomethyl glucose was isolated which was shown to be 2-methyl glucose. Since the monomethyl dicellulose does not give the viscose reaction it is concluded that the hydroxyl group in the 2 position in a glucopyranose residue of cellulose is that concerned in the viscose reaction. Subsequently this author showed that position 2 in glucose diethyl mercaptal appeared to be the most reactive. It is concluded therefore that the results presented in this section of the thesis are in agreement with Lieser's conclusions, inasmuch as soda cellulose is the precursor of xanthate formation, although they cannot be reconciled with the results of Piwonka. In the latter case the possibility exists that the copper present exerts a directive influence on the entering methoxyl groups although the reason for this anomaly is not clear.

It is worthy of note that in the experiments described in this thesis no evidence is forthcoming as to the entry of a methoxyl residue into the primary alcoholic groups present in either amylose or cellulose. In both cases position 2 appears to be involved.

Cellobiose and α - and β -methylglucosides undergo substitution at the primary alcoholic residues so that the theory (45) that in cellulose the $-\text{CH}_2\text{OH}$ groups are engaged in "secondary valency" formation with adjoining chains is strengthened by these observations. This is also probable in the case of amylose but here it must be admitted that in maltose, the fundamental building unit, both position 2 and 6 are involved in alkali compound formation.

E X P E R I M E N T A L .

TYPICAL PREPARATION OF POTASH-CELLULOSE.

The addition product was prepared by the method employed by Percival, Guthbertson and Hibbert (6).

To a quantity (5 gm.) of best quality surgical cotton, teased out into small pieces, 35% aqueous potassium hydroxide solution (75 c.c.) was added. The flask was securely corked and set aside for four hours. The product was then filtered off and as much as possible of the adhering alkali removed by pressure. It was next quickly transferred to a beaker containing alcohol (100 c.c.) with which it was thoroughly mixed and finally filtered off and dried over phosphorus pentoxide in a vacuum desiccator.

Yield 6 grams.

Analysis:-

The alkali content was determined by titration with acid in the usual way.

Found: KOH, 24.1%

calculated for a 1:1 addition compound $C_6H_{10}O_5$. KOH

KOH, 25.7%

TYPICAL METHYLATION OF POTASH-CELLULOSE.

The dry potassium hydroxide compound (5 gm.) was cut up into small pieces and placed in a flask fitted with a stirrer and quickly covered with dimethyl sulphate (120 c.c.) which had been previously neutralised with anhydrous potassium carbonate. The flask was then surrounded by a water bath which was maintained at 60°C. and the contents vigorously stirred till the methyl sulphate became just acid to litmus. The period required was 15 minutes. After cooling in ice the liquid was decanted off and the residue thoroughly washed with acetone to free it of dimethyl sulphate. The inorganic impurities were removed by washing with hot water (100 c.c. at 90°C.) and after pressing out as much of the water as possible from the product the methylated cellulose was dried overnight over phosphorus pentoxide in a vacuum desiccator.

Yield 3.6 grams.

The product was a white, fibrous substance similar in appearance to the original cotton but with a slightly harder feel.

Analysis:-

Found: OMe, 12.1%

calculated for a monomethyl cellulose $C_7H_{12}O_5$

 OMe, 17.6%

HYDROLYSIS OF METHYLATED CELLULOSE.

The above methylated product was combined with that obtained from another experiment and hydrolysed by the method of Hawerth and Machemer (36). The combined products (8 gm.) were cut into small pieces and vigorously stirred with cold concentrated hydrochloric acid for two hours at room temperature. The mixture was then cooled to zero and saturated with hydrochloric acid gas. In this way the solid was completely dissolved to give a very viscous yellow solution which was further saturated with gas at -20°C . This solution (flask closed by calcium chloride tube) was kept overnight surrounded by ice water and then again saturated with hydrochloric acid gas at -20°C . and kept overnight. This was repeated a third time. Thereafter the temperature was allowed to rise slowly and as much as possible of the hydrochloric acid removed first, by aeration and then by careful heating on a water bath at $25-30^{\circ}\text{C}$. under diminished pressure. The residual acid was then neutralised with silver carbonate and after thorough extraction of the silver residues with hot water the aqueous solution was subjected to preferential fermentation with yeast, for a period of six days. The yeast was filtered off and the filtrate taken to dryness under reduced pressure at 45°C .

GLUCOSIDE FORMATION.

The dried residue was extracted several times with dry methyl alcohol and then sufficient methyl alcoholic-hydrogen chloride added to the combined extracts to bring the acid concentration up to 5%. The glucoside mixture was refluxed till it no longer had a reducing action towards Fehling's solution. The period required being six hours.

The free mineral acid was neutralised with silver carbonate and the solution filtered. The silver residues were then thoroughly extracted with hot methyl alcohol and the extracts added to the original filtrate and the whole carefully evaporated to dryness at 35-40°C. under reduced pressure. The resulting product was a clear brown, non-reducing syrup.

Yield 3.4 grams.

ACETYLATION OF THE GLUCOSIDE.

The syrup (3.4 gm.) was dissolved in pyridine (50 c.c.) and acetylated by heating with acetic anhydride (25 c.c.) at 65°C. for 2½ hours and allowing the mixture to stand at room temperature for two days. The acetylation mixture was poured into a large volume of cold

water and the product extracted with chloroform (300 c.c.). After washing with sulphuric acid and neutralising the free acid with solid sodium bicarbonate the chloroform extract was taken to dryness in the usual way.

The acetate syrup was then fractionated by distillation in a high vacuum. Careful separation of the distillates resulted in three fractions being isolated between 140° and 210°C.

Fraction No:	Temp. °C.	Pressure. mms.	Yield. gm.	Methoxyl. %	Acetyl. %	CHCl ₃ D. 20°C.
A	140-150	0.03	0.14	30.8	29.9	51.6
B	185-195	0.03	1.96	18.8	38.5	77.0
C	195-210	0.08	0.72	11.3	44.6	-

There remained a residue (1.3 gm.) which would not distill even at 250°C.

FRACTION - A.

This fraction was obtained as a clear mobile liquid. The poor yield prevented a detailed examination.

Analysis:

Found: OMe, 30.8%, CH₃CO, 29.9%

calculated for a dimethyl diacetyl methylglucoside C₁₃H₂₂O₈

OMe, 30.4%, CH₃CO, 28.1%

From the analytical results it is apparent that this fraction consists mainly of a dimethyl diacetyl methylglucoside and further work will be required to elucidate its structure.

FRACTION - B.

The syrup obtained as the second fraction was a clear colourless syrup.

Found: OMe, 18.8%, CH₃CO, 38.5%

calculated for a monomethyl triacetyl methyl-glucoside C₁₄H₂₂O₉

OMe, 18.6% CH₃CO, 38.6%

A portion (1.2 gm.) of the syrup was deacetylated by Zemplén's (37) method. A cold dry methyl alcoholic solution (10 c.c.) of sodium (0.32 gm.) was added to a cold chloroform solution (20 c.c.) of the syrup and the whole immersed in a basin of cold water. At the end of three hours the solution was acidified and the deacetylated sugar extracted with water in the usual manner. The aqueous extract was then hydrolysed by adding sufficient sulphuric acid to bring the acid concentration of the solution to 1.5 N. and then heated at 95°C. till constant rotation was obtained.

Time	Reading	$\left[\alpha \right]_{D}^{20}$
0 mins	1.80	+71.7°
1 hour	1.60	63.6°
2 hours	1.51	59.7°
3 hours	1.45	57.7°
4 hours	1.38	53.7°
6 hours	1.35	53.5°

Constancy of rotation was obtained in six hours. The mineral acid was neutralised with barium carbonate and after the removal of the insoluble barium salts and thorough leaching of the mass with hot water, the aqueous extracts were taken to dryness at 40°C. under pressure. The residual mass, after several treatments with a benzene alcohol mixture, was thoroughly extracted with alcohol and again taken to dryness. Last traces of solvent were removed on a high vacuum pump at 60°C.

A quantity of this syrup (0.3 gm.) was subjected to osazone formation. Phenylhydrazine (1.0 c.c.) in acetic acid (1.5 c.c.), sodium acetate (0.5 gm.) and a small quantity of sodium bisulphite (38) were added to an aqueous solution (15 c.c.) of the syrup and the mixture heated at 95°C. Within an hour a precipitate had formed which was filtered off and washed with dilute acetic acid and water and dried in vacuo. Yield 0.05 gm.

OMe nil (crude)

Recrystallisation was effected from aqueous pyridine.

m.p. 197°C.

On admixture with a known sample of glucosazone the phenylosazone gave no depression in the melting point and was considered identical.

The remaining portion (0.3 gm.) of the hydrolysed syrup was acetylated with pyridine (25 c.c.) and acetic anhydride (12.5 c.c.) by heating at 65°C. for 3 hours. After standing for two days at room temperature the acetylation product was worked up as before to give a dark coloured syrup

Yield 0.38 grams.

Analysis:—

Found: OMe, 9.94%, CH₃CO, 45.6%

Calculated for a monomethyl tetra-acetyl glucose C₁₅H₂₂O₁₀

OMe, 8.57% CH₃CO, 47.5%
 $[\alpha]_{D}^{17^{\circ}C.} + 51.7^{\circ}$ in acetone (c= 0.60)

ATTEMPTED ISOLATION OF 2-METHYLGLUCOSE PHENYLHYDRAZONE.

An attempt was made to isolate the phenylhydrazone of the sugar.

A portion (0.62 gm.) of the acetate was deacetylated and hydrolysed as before and the syrup extracted with alcohol from the dried hydrolysis residue. This was taken to dryness and all traces of alcohol removed on a high vacuum pump. A light brown syrup was obtained.

The syrup (0.28 gm.) was dissolved in the minimum of water (0.05 c.c.) and pure phenylhydrazine (0.5 c.c) and acetic acid (3 drops) were added and after shaking the mixture was set aside for 4 days. No trace of the

phenylhydrazone could be found and it was concluded that the sodium acetate known to be present in large quantity (50%) was detrimental to the formation of the hydrazone. Munro (34) found that the presence of sodium acetate retarded the formation of 2-methylglucose phenylhydrazone.

FRACTION -C.

The syrup obtained in this fraction was clear and colourless. From the analytical results it is apparently methylglucoside derived from the glucose which had escaped removal during the fermentation process earlier.

The syrup (0.58 gm.) was deacetylated by dissolving in chloroform (10 c.c.) and adding to a cold solution of sodium (0.2 gm.) in methyl alcohol (10 c.c.). After remaining immersed in a basin of water for three hours the deacetylated sugar was extracted with water.

A sufficient quantity of sulphuric acid was added to the aqueous extract to make the acid concentration 1.5 N. and the mixture heated at 95°C. till constant rotation was attained. A period of seven hours was required.

Time.	Reading.	$\left[\alpha \right]_{D.}^{20^{\circ}}$
0 mins	1.10	75.1°
2 hours	0.85	58.2°
4 hours	0.75	51.2°
6 hours	0.68	46.4°
8 hours	0.65	44.4°

The hydrolysis solution was neutralised with barium carbonate. The barium residues were filtered off and after treatment of the residue with hot water the combined aqueous solutions were taken to dryness and the hydrolysed syrup extracted from the dried mass with hot alcohol.

The alcoholic extract was next evaporated to dryness under diminished pressure and the product dissolved in water (20 c.c.). Phenylhydrazine (1.0 c.c.) in acetic acid (1.5 c.c.), crystalline sodium acetate (0.3 gm.) and sodium bisulphite (38) were added and the mixture heated on a water bath at 90°C. Within a short time a bright yellow osazone was precipitated.

Yield 0.07 grams.

Analysis:-

Found: OMe, nil.

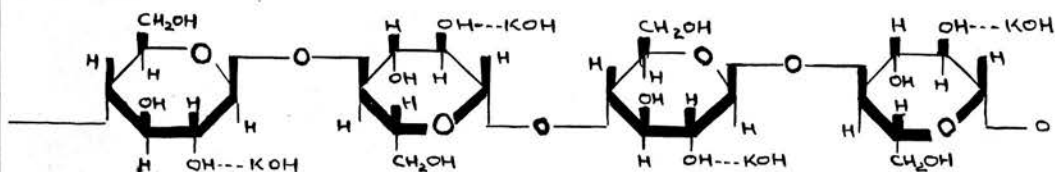
The osazone was recrystallised from aqueous pyridine.

m.p. 203°C.

A mixed melting point with glucosazone showed no depression.

S U M M A R Y.

1. Mild methylation of potash cellulose gave rise to a partially methylated product.
2. Hydrolysis of the methylated cellulose product, followed by glucoside formation, acetylation and fractionation gave rise to a di- and a mono-methyl methylglucoside acetate.
3. A detailed examination of the dimethyl methylglucoside was not made.
4. The monomethyl methylglucoside acetate was shown to be derived from 2-methyl glucose by identification of the osazone as glucosazone.
5. Evidence is therefore presented from a consideration of facts that "potash cellulose" consists mainly of the compound having the structure.



6. This is in agreement with the work of Lieser on the structure of the cellulose xanthates but is not consistent with the results obtained by Piwonka from his work on the cupri soda-cellulose compounds.

B I B L I O G R A P H Y.

- (1).Hubner..... J.Soc. Dyers & Col.,1911,27,
- (2).Gladstone..... J.C.S., 1852, 5, 17.
- (3).Thiele..... chem. Z., 1906, 30, 584.
- (4).Vieweg..... Ber., 1907, 40, 3876.
- (5).Norman..... chem, Z., 1906, 30, 584.
- (6).Percival,Cuthbertson
and Hibbert..... J.A.C.S., 1930, 52, 3257.
- (7).Rassow and Wolf..... Ber., 1929, 62, 2949.
- (8).Vieweg.....
- (9).Rassow and Wadewitz.. J.prakt. Chem., 1923,106,266.
- (10).Heuser and Niethammer.Cellulosechemie,1925,6, 13.
- (11).Karrer..... Cellulosechemie,1921,2,125.
- (12).Katz..... Cellulosechemie,1925,6, 35.
- (13).Dehnert and Koenig... Cellulosechemie,1924,5,107.
- (14).Dehnert and Koenig... Cellulosechemie,1925,6, 1.
- (15).d'Ans and Jaeger..... Cellulosechemie,1925,6,137.
- (16).Hess..... Ann., 1923, 435, 1.
- (17).Hess..... Z. angew. Chem.,1925,38,320.
- (18).Knecht and Platt..... J.Soc.Dyers & Col.,1925,41,53.
- (19).Liepatoff..... J. Russ.Phys-chem Soc.,1925,57,48.
- (20).Katz..... Z.Elektrochem.,1925,31,157.
- (21).Schwarze..... Dissertation - Leipzig, 1929.
- (22).Leighton..... J.Physic.Chem.,1916,20, 32.

- (23). Coward and Spencer..... J. Textile Inst., 1923, 14, T32.
- (24). Millar..... Ber., 1907, 40, 4903.
- (25). Millar..... Ber., 1908, 41, 4292.
- (26). Millar..... Ber., 1910, 43, 3430.
- (27). Millar..... Ber., 1911, 44, 728.
- (28). Heuser..... Z. angew. chemie., 1924, 37, 1010.
- (29). Clark..... Tech. Assoc. paper Series X p.43.
- (30). Vieweg..... Cellulosechemie, 1925, 6, 35.
- (31). Herzog..... Z. physik., 1926, 121, 136.
- (32). Heuser and Bartunck..... Cellulosechemie, 1925, 6, 14.
- (33). Hubner and Tetlscher..... J. Soc. Chem. Ind. 1909, 28, 641.
- (34). Munro..... Thesis, 1936, Edinburgh Univ.
- (35). Piwonka..... Ber., 1936, 69, 1965.
- (36). Haworth and Machemer.... J.C.S., 1932, 2270.
- (37). Zemplén and Kunz..... Ber., 1923, 56, 1705.
- (38). Hamilton..... J.A.C.S., 1934, 56, 487.
- (39). Lieser Ann., 1932, 495, 235.
- (40). Lieser Ann., 1929, 470, 104.
- (41) Lieser and Leckzyck Ann., 1934, 511, 137.
Ann., 1936, 522, 56.
- (42). Lieser and Hackl Ann., 1934, 511, 128.
- (43). Lieser Ann., 1930, 483, 132.
- (44). Traube, Piwonka and
Funk Ber., 1935, 69, 14.
- (45) Cox Ann., Reports, 1937, 189.

THE STRUCTURE OF THE SUGAR ALKALI COMPOUNDS.

THE STRUCTURE OF THE SUGAR ALKALI COMPOUNDS.

It is evident from the compounds isolated and discussed in this thesis that under certain conditions a chemical combination occurs between the alkalis and the sugars and it remains to consider the probable nature of the linkages involved.

It has been shown in a previous section that an alcoholate type of structure was unlikely since it was impossible to form an ether from these compounds by replacement of the alkali groups such as methyl or ethyl. Further MacKenzie and Quin (1) were unable to isolate any identifiable methylated products from the action of methyl sulphate on a lime sucrose compound.

It should be noted in passing that recently Muskat (2) has isolated a different kind of sugar alkali compound which can be made to react with methyl iodide to give an almost quantitative yield of methylated sugar. But these compounds were prepared by the rather drastic method of adding metallic potassium to solutions of sugars in liquid ammonia. These appear to have an alcoholate structure. This, however, would not apply to the sugar alkali compounds under discussion since Ritchie (3) showed that methylation of

similar alkali sugar compounds with methyl iodide was unsatisfactory, a 5% yield of a very inferior product being the best obtainable.

In the present work it was shown that by a single treatment with dry neutral dimethyl sulphate upon the sugar alkali compounds under mild conditions and for short duration it was possible to replace each alkali residue shown previously to be present by analysis, by a methyl group. The amount of unchanged compound is, however, very considerable, the conversion being about 10% in most cases. Although this is a practical disadvantage it shows that the reaction is not a case of methylation comparable with the usual standard method. This was further investigated by Percival (4) who treated a mixture of mono-molecular proportions of glucose and potassium hydroxide with dimethyl sulphate under the same conditions as were in force for methylation of the addition compounds. The yield of methyglucoside never amounted to more than 0.5% of the glucose taken. Further support for the addition type of compound was given by the isolation of compounds of the type $C_6H_{12}O_6.NaOR$ (4, 5) from glucose and sodium alkoxide in the complete absence of moisture appeared to support this addition theory.

The great instability of these addition compounds suggests that the union of the potassium hydroxide and sugar is very feeble and may be considered to be due to some form of co-ordination. Since the formation of a co-ordinate (or electrovalent) linkage requires the presence of two atoms, one with a pair of unshared electrons and the other with a deficiency of such a pair the requisite conditions are present in the hydroxyl group, a typical associating group, in both sugar and alkali. Considering the case of glucose it is seen that the inorganic residue is attached to the reducing group and it is improbable that the oxygen atom of that group can act as a donor of electrons to the metal atom concerned since it has been shown that in glucosidyl union the KOH group ceases to be associated with the blocked reducing group and becomes attracted instead to the hydroxyl in another position. The oxygen of the sugar hydroxyl group then does not act as a donor of electrons but the hydrogen of this group appears to become an acceptor of electrons from the oxygen atom of the alkali hydroxide.

This is in complete agreement with the well-known fact that alkaline hydroxides appear to form very stable monohydrates which is attributed to hydration of the

negatively charged hydroxyl ion, $\text{HOH} \leftarrow \text{OH}'$, the oxygen atom of which acts as a powerful donor of electrons (6).

In these co-ordination compounds with linkage through the hydrogen atom of the carbohydrate hydroxyl, the hydrogen atom appears to take up a maximum of four valence electrons, but in the light of recent developments this is doubtful and an alternative system may be provided by the theory of resonance. (Ritchie - 3).

Some other explanation may emerge later and in view of the somewhat negative evidence available it appears inadvisable to attempt a precise definition of the type of linkage involved and for the time being it is proposed to represent the union between carbohydrates and alkali hydroxides by means of a dotted line as originally used by Werner to denote residual valencies.

B I B L I O G R A P H Y.

- (1) MacKenzie and Quin J.C.S., 1929, 951.
- (2) Muskat J.A.C.S., 1934, 56,
693, and 2449.
- (3) Ritchie Thesis, 1936, Edin-
burgh University.
- (4) Percival J.C.S., 1934, 1160.
- (5) Zemplén and Kunz Ber., 1923, 56, 1705.
- (6) Sidgwick "Electronic Theory of
"Valency", Oxford,
1934.

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