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Title.

A Study of Certain Addition Compounds of the Carbohydrates

A STUDY OF CERTAIN ADDITION COMPOUNDS OF THE CARBOHYDRATES

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Studies on the Addition Compounds of the Carbohydrates

In the literature may be found many references to compounds formed by the interaction of sugars with metallic oxides and hydroxides, more especially those of the alkali and alkaline earth metals. Such compounds are of more than theoretical interest, since those of calcium and strontium hydroxide find technical application in sugar refining. As early as 1838 Peligot (1) published a paper on the compounds of sucrose with various metallic oxides in which he gave analyses of several which he had prepared. In spite of the fact that such compounds have thus been known and studied over the period of a century, their constitution has not yet been satisfactorily determined.

Previous investigation of this problem has been largely concerned with determining the stoicheiometric relationships between the organic and inorganic constituents, and a large number of compounds in which there does exist a definite stoicheiometric

relationship between the two components have been recorded. Such determinations however do not provide much evidence as to structure. To take, for example, the typical case of sodium glucosate, from the evidence put forward it is uncertain by which formula this compound would be better represented,

$$C_6H_{12}O_6,NaOH \quad \text{or} \quad C_6H_{11}O_6Na.$$

Several later workers have preferred to approach the subject from a physicochemical point of view, regarding the sugars as weak acids.

Madsen (2), from determinations of the velocity of hydrolysis of ethyl acetate by sodium hydroxide in the presence of glucose at different temperatures, calculated the heat of neutralisation of glucose and sodium hydroxide to be 5340 calories. He assumed glucose to be a weak monobasic acid.

Hirsch and Schlags (3), however, carried out conductivity measurements on solutions of sodium hydroxide in which various sugars were dissolved, the conductivity being decreased due to compound formation. They investigated the cases of glucose, fructose, maltose, sucrose, and lactose, and came to

the conclusion that each sugar contained more than one acidic group in the molecule, the simplest assumption to make being that they were dibasic. The following dissociation constants are given:

	$K_1 \times 10^{-13}$	$K_2 \times 10^{-14}$
Glucose:	7.8	1.54
Fructose:	20.3	1.56
Maltose:	11.6	7.7
Sucrose:	3.1	3.0
Lactose:	10.5	3.6

These measurements were carried out at $25^{\circ}C$.

Stearn (4) goes much further than this. He gives conductometric data for the same sugars in sodium hydroxide solution, and states that in twice normal sodium hydroxide they act as polybasic acids with at least five ionising hydrogen atoms.

Urban and Shaffer (5), however, state that electrometric titrations of glucose, fructose, and sucrose with sodium hydroxide using the hydrogen electrode indicate these sugars to be behaving as dibasic acids with the following dissociation constants:

	pK_1'	pK_2'
Glucose:	12.09	13.85
Fructose:	11.68	13.24
Sucrose:	12.60	13.52

These measurements were carried out at 25°C. They also add that at high alkalinity a third acidic group seems to come into play.

Urban and Williams (6) confirmed the above results by the use of the glass electrode, but could find no trace of a third dissociation constant before p_H 13.6.

While many of the alkaline earth sugar compounds are definite crystalline solids which are insoluble in water and stable towards it, 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. The above conductivity experiments do however indicate that in spite of this some combination does take place in aqueous solution.

This was likewise shown by Groot (7) who observed that the maximum depression in the specific

rotation of glucose solution in the presence of sodium or potassium hydroxide was obtained when the alkali and the sugar were present in approximately molecular proportions. From measurements of the rate of decline of rotation at different concentrations, he concluded that the dissociation constant of glucose as an acid was $K = 8.6 \times 10^{-13}$ at 25°C., the decline of rotation being accounted for by the initial formation of an unstable compound $C_6H_{11}O_6K$, followed by Lobry de Bruyn - van Eckenstein transformations.

Such methods as those described fail to indicate the cause of the apparent acidity and the point at which it arises in the molecule, although Michaelis and Rona (8), on the basis of potentiometric measurements of hydrogen-ion concentrations of solutions of alkali hydroxides and sugars, suggest the possibility that the acidity is due to the presence of enolic forms -CH(OH)=C(OH)-.

Hirsch and Schlags (3) however consider that their results are not consistent with this theory, which in any case could only be applied to the reducing sugars, and would furnish no explanation of

the extensive series of compounds formed by sucrose with alkali and alkaline earth hydroxides.

Gabryelski (9) found that the presence of hydroxyl ions altered the absorption spectrum of glucose or galactose. The alteration took place before any pigmentation occurred and, if the solution was immediately neutralised the original spectrum was regenerated. He considered that products containing aldehydic complexes were formed in the solution.

It will be seen that no definite ideas as to the structure of the compounds formed can be obtained from the foregoing physicochemical determinations, which in general merely show that sugars are capable of removing alkali or hydroxyl ions from solution.

There are three main possibilities as to the nature of the products which have been considered.

First, they may not be definite chemical compounds at all. Some workers, especially those in the field of the polysaccharides ascribe the whole reaction to a process of adsorption. It is certainly true that, from an aqueous solution of e.g. sucrose and lime, precipitates of widely varying com-

position, in which no definite relationship between the constituents is apparent, may be thrown out by the addition of alcohol. On the other hand, the fact that over eighty authenticated compounds have been isolated from various carbohydrates and alkalies in which the constituents appear to be present in stoicheiometric proportions [see e.g. Mackenzie and Quin (10)] seems to point to definite chemical reaction and not merely adsorption or fortuitous precipitation of the metallic constituent along with the carbohydrate. In addition, when both the sugar and the hydroxide are soluble in the reaction medium simple mixing of the solutions is sufficient to precipitate the compound. This would appear to be a definite case of chemical reaction.

Two alternative suggestions as to the structure of these compounds have been put forward; they may be either substitution compounds comparable to those of the alcohols, e.g. sodium ethoxide, or they may be some type of addition compound, in which the metallic hydroxide is co-ordinated to some reactive point within the sugar molecule.

Hönig and Rosenfeld (11) claimed to have isolated the compound $C_6H_{11}O_6Na$ from glucose and sodium ethoxide in alcoholic solution. If this alcoholate formula is the correct one, then such a compound should prove capable of being used in synthetic experiments.

Zemplén and Kunz (12) pointed out that this did not appear to be the case, and drew attention to attempts by Skraup and Kremann (13) to condense tetra-acetyl glucosidyl chloride and tetra-acetyl galactosidyl chloride with this sodium-glucose compound in the hope of producing a disaccharide. This they were unable to do.

After thus casting doubt upon the alcoholate formula, Zemplén went on to show that actually an addition compound of the type $C_6H_{12}O_6,NaOC_2H_5$ appeared to be formed by glucose and sodium ethoxide, the presence of the ethoxide residue being established by a qualitative test.

Percival (14), by working with materials which had been thoroughly dried, confirmed the existence of this compound and of the corresponding one pre-

pared with sodium methoxide. He also explained the earlier results by stating that, in the presence of small traces of moisture, the compounds isolated contained no combined alcoholic residue owing to the hydrolysis of the sodium ethoxide. From glucose and potassium hydroxide in absolute alcoholic solution the compound $C_6H_{12}O_6$,KOH was obtained. This formula, derived by analogy from the sodium alkoxide compounds, was also supported by analytical data.

Thus it would appear that the compounds formed between sugars and alkali hydroxides are of the addition compound type. The fact that several sugars are capable of forming compounds with various metallic salts lends force to this idea. As examples of such compounds may be mentioned glucose-sodium chloride (15), sucrose-sodium iodide (16) and mannose-calcium chloride (17).

Before being able to decide what type of linkage is brought into play in these compounds, some information is required as to the position within the sugar molecule of the reactive points at which the inorganic constituents become attached.

It has frequently been assumed that, in the case of reducing sugars, the reducing group is actively concerned in the union. This assumption is however based rather on the fact that the reducing group is naturally the most reactive point within the molecule than on any direct evidence.

Marchlewski (18) was unable to obtain glucose phenylosazone from phenylhydrazine and potassium glucosate, and attributed this to the fact that the alkali metal was attached to the reducing group. This, however, is inconclusive, since, in the normal production of glucose phenylosazone from glucose and phenylhydrazine, acidification with acetic acid is necessary.

With a non-reducing sugar such as sucrose or a methylglucoside not even the above assumption can be made and the position is therefore completely obscure.

It was, however, shown by Percival (14) that, by the selective action of a reagent such as methyl 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 potassium "glucosate" $C_6H_{12}O_6$,KOH, the inorganic constituent really did appear to be intimately connected with the reducing group, the evidence being based on the isolation of crystalline tetra-acetyl β-methyl-glucoside.

The purpose of this work is to extend this method of examination to the potassium hydroxide compounds of other sugars and in particular the disaccharides cellobiose and lactose.

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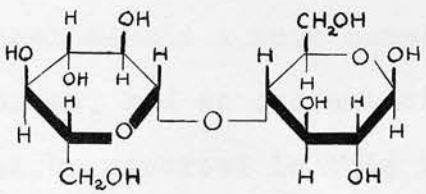
PART I

The Addition Compounds of Cellobiose and Potassium Hydroxide

The Addition Compounds of Cellobiose and Potassium Hydroxide

The first sugar chosen for investigation was cellobiose. This was selected as it has the structure of glucose β-glucoside:



Cellobiose

As such it is important as the fundamental unit in cellulose, and a knowledge of the structure of the alkali compounds of this sugar might possibly be important in connection with the constitution of alkali-cellulose.

There are but few references to compounds of cellobiose with metallic hydroxides. The isolation of the potassium compound was described by Haworth and Hirst (1). This preparation was however incidental to the deacetylation of cellobiose octa-acetate

and no detailed examination of the compound was carried out.

The action of weak alcoholic potassium hydroxide on cellobiose was examined by Percival (2). He found that a compound of the type $C_{12}H_{22}O_{11}$,KOH appeared to be formed in which the potassium hydroxide was closely associated with the reducing group of the sugar.

With stronger alkali a more complex compound appeared to be formed, and an account of the examination of this will be reported in this thesis.

Before proceeding to isolate the compounds formed between cellobiose and alkalies, and investigate their structure, it is necessary to know the proportions in which the two constituents combine. This is decided by a titration method as follows.

To a measured amount of an alcoholic solution of the sugar of known strength, a known volume of standard alcoholic potassium hydroxide is added. A fine white precipitate of the sugar alkali compound is immediately thrown out of solution. This is filtered off through a Gooch crucible, and a portion of the filtrate titrated against standard acid.

From the decrease in alkalinity of the solution, the amount of potassium hydroxide withdrawn and held in combination by the sugar can be calculated. Two assumptions have to be made; first that, on the mixing of the sugar and alkaline solutions, no appreciable volume changes take place; and second that the sugar alkali compound is insoluble in the equilibrium mixture. The validity of this last assumption varies with different sugars, and thus the figures given by this method are only to be regarded as fairly close approximations.

If the compound in the crucible is drained, washed with a little alcohol to remove adhering liquid, and dissolved in water, titration against acid then gives a direct value for the combined alkali. This figure is however less consistent than that given by the previous indirect method, and is probably less accurate, due to partial decomposition of the sugar-alkali compound during the washing with alcohol.

Figures obtained by applying the above methods to cellobiose are quoted by Percival (2) as follows

in columns I-IV. Columns V and VI are two additional determinations which were made to verify the values given for the higher concentration of alkali.

	I	II	III	IV	V	VI
Concⁿ. of Cellobiose %	1.8	1.9	1.49	0.88	0.77	0.57
Concⁿ of KOH — Initial Normality	0.075	0.15	0.65	0.76	0.742	0.839
Concⁿ of KOH — Final Normality	0.02	0.08	0.58	0.69	0.697	0.807
KOH in g. combined with 100 g. Cellobiose — Indirect Method	17.1	21.4	26.0	32.9	33.2	32.5
KOH in g. combined with 100 g. Cellobiose — Direct Method	16.0	21.4	19.5	23.0	25.1	25.7

To form a compound of the type $C_{12}H_{22}O_{11},KOH$, 100 g. of cellobiose would require to combine with 16.4 g. potassium hydroxide. For $C_{12}H_{22}O_{11},2KOH$ the figure is 32.8 g. potassium hydroxide.

Thus it would appear that, with alkali exceeding approximately 0.7 in normality the compound formed is of the latter type, whilst for lower concentrations mixtures are produced.

The compound formed in dilute alkali and approximating to the former type has been examined by Percival (2) and shown to have the potassium hydroxide intimately connected with the reducing group.

The method for determining the structure of these compounds depends on the introduction of a stable group in place of the more loosely attached alkali. This is accomplished by methylation of the isolated compound under mild conditions. The reagent used is dry methyl sulphate which has been made neutral by the addition of anhydrous potassium carbonate. A single application of the reagent is employed and the time of reaction kept as short as possible. Under these conditions it is found that for each alkali residue originally combined with the sugar a corresponding number of methyl groups is introduced into the sugar molecule. In order to minimise the possibility of the alkali residue migrating to a fresh position in the sugar molecule during the course of the reaction, care is taken to have both the reacting substances as dry as possible. If however water or other ionising solvent were present, the method does not necessarily fail, as it can still be expected to indicate those sugar hydroxyl groups of maximal acidity, since, whether they are closely bound to alkali or not, the centres of acidity are not likely to change during the operation.

The process for determining the positions in the cellobiose molecule of the alkali constituents in the cellobiose potassium hydroxide complex of formula $C_{12}H_{22}O_{11}$,2KOH was as follows.

The cellobiose was prepared in the form of the octa-acetate by the simultaneous hydrolysis and acetylation of cellulose, the method used being that of Haworth,Hirst, Streight, Thomas and Webb (3). The cellobiose potassium hydroxide compound was formed by making the cellobiose octa-acetate into a paste with absolute alcohol and adding an excess of absolute alcoholic potassium hydroxide. This caused the deacetylation of the octa-acetate and the liberation of the cellobiose which immediately combined with the excess of potassium hydroxide to form an insoluble compound.

After isolation the compound was thoroughly dried and subjected to a mild treatment with neutral dimethyl sulphate. Treatment of the product in alcoholic solution with potassium hydroxide removed unsubstituted cellobiose by precipitation. Acetylation of the residue then yielded a mixture of hepta-

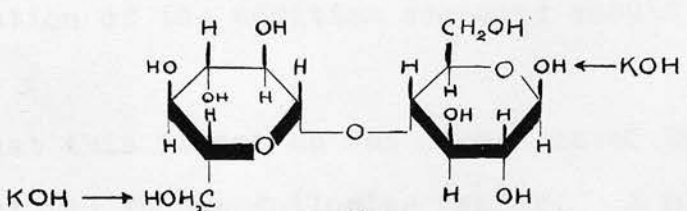
acetyl β-methylcellobioside and a hexa-acetyl monomethyl methylcellobioside, the respective amounts obtained under the best conditions being about 12% and 27% of the theoretical based on the quantity of cellobiose octa-acetate taken. The former was removed by crystallisation from alcohol, and the syrupy remainder was deacetylated and then hydrolysed with dilute acid. This gave a mixture of glucose and a monomethyl glucose, from which the former was removed by precipitation with potassium hydroxide in alcoholic solution. The methyl glucose was found to give a phenylosazone which proved to be identical with the phenylosazone of 6-methyl glucose.

Methylation of the cellobiose potassium hydroxide compound approximating in composition to $C_{12}H_{22}O_{11},2KOH$ thus gave a monomethyl methylcellobioside in which the non-glucosidic methyl group was situated in the 6-position of one of the two glucopyranose units comprising cellobiose. From the above series of reactions it was not possible to tell into which unit this methyl group had been introduced. This was due to the fact that the two glucopyranose halves of the cellobiose molecule

could not be distinguished after hydrolysis, since the glucosidic methyl group, which had also been introduced, was simultaneously removed.

The β-methylcellobioside obtained as another product of the methylation would be derived from cellobiose potassium hydroxide compound of the type $C_{12}H_{22}O_{11}$,KOH, a proportion of which would inevitably be present in the initial material.

From the foregoing facts, therefore, the deduction is made that cellobiose combines with two molecular proportions of potassium hydroxide to form a compound in which one of the alkali residues is closely connected with the reducing group; the other is located at the primary alcoholic residue on the terminal carbon atom of one of the glucopyranose units in the molecule. These positions are indicated in the following formulae:



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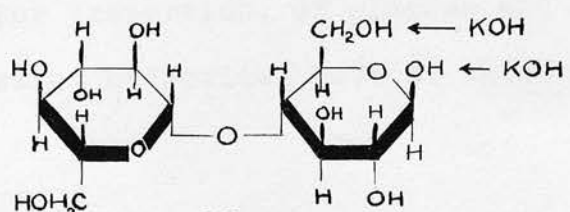

II

Since one of the standard methods for methylating a sugar consists in treating it with alkali and methyl sulphate, it might be argued that the results obtained above are dependent merely on this fact; or, in other words, that the methylated products obtained are not concerned with the presence of sugar alkali compounds in the starting material, their formation being due merely to the presence of some free potassium hydroxide. The formation of partly substituted sugars in preference to that of more fully methylated products would then be accounted for by the mildness of the conditions employed, and the absence of a solvent.

If this is the case, then, by methylating a mechanical mixture of sugar and alkali in the same proportions as those in which they are found in combination, products identical to those produced by the methylation of the addition compound should be obtained.

That this is not so was demonstrated by Percival (4) in the following manner. A mixture, in monomolecular proportion, of glucose and finely powdered potassium hydroxide, both of which had

previously been dried in a vacuum over phosphorus pentoxide, was methylated with neutral dimethyl sulphate under the same conditions as were in force for the methylation of the addition compounds. Only a very small yield of methylglucoside could be isolated, and this never amounted to more than 0.5% of the weight of glucose taken. This compared with a conversion of about 20% when the addition compound of glucose and potassium hydroxide was used.

It may also be mentioned that, under the same conditions, dimethyl sulphate had no action on cellobiose in the absence of alkali, the sugar being recovered unchanged from the reaction mixture.

An attempt was made to carry out the methylation of cellobiose-potassium hydroxide compound using methyl iodide instead of methyl sulphate. In this case however conversion to an acetylated syrup containing 8% of methoxyl took place only to the extent of 5%, as compared with a yield of over 30% (based on the methoxyl content) when methyl sulphate was employed. Methyl iodide therefore did not appear to be so efficient a reagent for this work.

E_X_P_E_R_I_M_E_N_T_A_L

Preparation of Cellobiose Octa-acetate

This was prepared after the method of Haworth, Hirst, Streight, Thomas and Webb (3). The mixture for acetylation was made up by adding concentrated sulphuric acid (14 c.c.) to acetic anhydride (100 c.c.), the whole being kept well cooled. Sheets of pure filter paper (25 g.) were cut up into pieces 1 cm. square, and added in small portions to the acetylation mixture. The whole was kept constantly stirred, and surrounded by a freezing mixture in order to keep the temperature below 20°. Stirring was continued until the paper was reduced to a mass of pulp, and the reaction vessel was then heated in a boiling water bath. The thick paste rapidly darkened and became mobile. When it reached the stage of a thin dark red liquid, and was on the point of turning black, it was poured into 1500 c.c. cold water. A yellow precipitate of the crude cellobiose octa-acetate separated after about 10-15

minutes. After standing overnight under water, this was filtered, washed with water, and dried. Purification was effected by boiling under reflux with 90% alcohol for half an hour, filtering while hot, and allowing to cool. The octa-acetate separated in small colourless needles which were washed with dilute alcohol and dried in an oven at 40°, m.p. 226°. The average yield was about 25% of the weight of the paper taken, but, until one had gained experience in the method, this was subject to considerable variation, owing to the fact that determination of the appropriate moment at which to check the acetolysis depended upon the judgement of colour changes.

The Determination of Combined Alkali

Cellobiose (0.2010 g.) prepared by Zemplén's method (5) from the octa-acetate, was dissolved in 90% alcohol (6 c.c.). To this solution standard alcoholic potassium hydroxide (20 c.c.) was added, and the mixture allowed to stand for 10 minutes. The precipitated cellobiose potassium hydroxide compound was removed by filtration through a Gooch crucible,

and an aliquot portion of the filtrate titrated against standard sulphuric acid, using phenolphthalein as indicator.

The precipitate in the crucible was drained, washed with a minimum quantity of absolute alcohol, dissolved in water and the solution titrated.

Results:

I. Indirect Method

2 c.c. standard alcoholic potassium hydroxide required 19.30 c.c. N/10-sulphuric acid for neutralisation.

Hence the sugar-alkali reaction mixture was initially equivalent to 193 c.c. N/10-sulphuric acid.

3 c.c. filtrate required 20.9 c.c. N/10-sulphuric acid for neutralisation.

Hence the sugar alkali reaction mixture was finally equivalent to

$\frac{26}{3} \times 20.9 = 181.1$ c.c. N/10-sulphuric acid.

Hence the alkali withdrawn from solution by 0.2010 g. cellobiose was equivalent to 11.9 c.c. N/10-sulphuric acid.

Hence 100 g. cellobiose would withdraw

$\frac{11.9}{0.201} \times \frac{56.1}{10} = 33.2$ g. potassium hydroxide

II. Direct Method

9.00 c.c. of N/10-sulphuric acid were required for the direct titration of the dissolved precipitate. Whence 100 g. cellobiose had combined with 25.1 g. potassium hydroxide.

A similar experiment in which a greater excess of slightly stronger alkali was used gave figures of 32.5 g. and 25.7 g. for the two respective methods. (see Table p.16.). The theoretical figure required for a compound, $C_{12}H_{22}O_{11},2KOH$ is 32.8 g.

Typical Preparation of Cellobiose-Potassium Hydroxide Compound

Dry cellobiose octa-acetate (20 g.) was made into a paste with absolute alcohol (40 c.c.). To the mass was added with agitation potassium hydroxide (40 g.) dissolved in absolute alcohol (175 c.c.), a solution of this high concentration being used to ensure the formation of a maximum proportion of the $C_{12}H_{22}O_{11},2KOH$ compound. Very strong alkali solu-

tions could not be used because of their destructive action on the sugar. The mixture was stirred in a closed flask for 2 hours, when the crystalline form of the octa-acetate had disappeared, and a white amorphous precipitate of the cellobiose alkali compound had taken its place. This was filtered rapidly at the pump, washed quickly with minimum quantities of absolute alcohol and dry ether, and dried over phosphorus pentoxide in a vacuum desiccator.

Yield 13 g.

The lumps of product crumbled on slight pressure to a white, amorphous, powdery solid, which was exceedingly deliquescent, and on short exposure to the atmosphere rapidly turned yellow, finally becoming a brown syrupy mass. It was found, however, that the dry compound could be preserved unchanged indefinitely in a desiccator.

A Typical Methylation of the Cellobiose-Potassium Hydroxide Compound

Dimethyl sulphate which had been neutralised with anhydrous potassium carbonate was used. The

finely powdered potassium compound obtained from cellobiose octa-acetate (9.5 g.) was placed in a litre flask fitted with a stirrer, and quickly covered with 40 c.c. of neutral dimethyl sulphate at room temperature. Vigorous stirring was carried on, while the flask was surrounded with a water bath which was maintained at 35-40° for 5 minutes and then raised to 70-75° for 10 minutes. The stirring was continued during the cooling of the reaction mixture to room temperature. The powdery solid coagulated and formed a viscous mass, which adhered to the sides of the flask. The clear supernatant liquid was decanted off, and the remaining solid, after being well washed with acetone, was boiled with a sufficient quantity of methyl alcohol to dissolve all the syrup. On cooling the solution deposited crystals of potassium methyl sulphate, and, after the removal of this, the clear solution was evaporated at 50° under reduced pressure to yield a reducing glass, which was dissolved in methyl alcohol (50 c.c.). A solution of potassium hydroxide (2 g.) in absolute alcohol (30 c.c.) was added, causing the immediate precipitation of the cellobiose potassium hydroxide

compound (3.5 g.). This was filtered off, quickly washed with alcohol and ether, and kept in a desiccator for a future methylation.

After ensuring that the further addition of alcoholic potassium hydroxide did not precipitate any more potassium cellobiosate, the solution was acidified with glacial acetic acid and evaporated to dryness at 50° under reduced pressure.

This yielded a mixture of potassium acetate and the methylated cellobiose, which was acetylated by heating with acetic anhydride (50 c.c.) and anhydrous sodium acetate (10 g.) at 100°C. for 1 hour. The acetylation mixture was allowed to cool, and then poured into cold water (250 c.c.). The solution was completely neutralised with solid sodium bicarbonate, and extracted thoroughly with chloroform (500 c.c.). The chloroform extracts were dried by addition of anhydrous sodium sulphate, and, after filtration, evaporated at 40-50° under diminished pressure. A syrup remained, and from the alcoholic solution of this crystals (0.5 g.) were obtained. On removal of alcohol from the

mother liquor a syrupy fraction (1.9 g.) was obtained.

The potassium cellobiosate recovered in the earlier part of the experiment was methylated and worked up in an identical manner to give further yields of the crystalline and the syrupy fractions.

Details of the yields in some of these preparations are as follows:

I. 9.5 G. of cellobiose octa-acetate were converted into the potassium hydroxide compound and methylated at 60° for 8 mins. and then at 70-75° for 5 mins.

Potassium hydroxide-cellobiose recovered = 3.5 g.
Crystalline fraction obtained ..... = 0.5 g.
Syrupy fraction obtained ..... = 1.9 g.

II. 3.5 G. of the potassium hydroxide-cellobiose recovered from (I) were methylated at 70° for 10 mins.

Potassium hydroxide-cellobiose recovered = 1.7 g.
Crystalline fraction obtained ..... = 0.16 g.
Syrupy fraction obtained ..... = 1.2 g.

III. 12 G. cellobiose octa-acetate were converted into the potassium hydroxide compound and methylated for 5 mins. at 35-40°, then for 5 mins. at 80-85° and finally for 5 mins. at 70°.

Potassium hydroxide-cellobiose recovered = 4.9 g.
Crystalline fraction obtained ..... = 1.45g.
Syrupy fraction obtained ..... = 3.2 g.

IV. 4.9 G. potassium hydroxide-cellobiose recovered from (III) were methylated for 5 mins. at 40°, then for 5 mins. at 80-85°, and finally for 5 mins. at 70°

Potassium hydroxide-cellobiose recovered = 4.3 g.
Crystalline fraction obtained ..... = 0.3 g.
Syrupy fraction obtained ..... = 0.9 g.

V. 4.3 G. potassium hydroxide-cellobiose recovered from (IV) were methylated for 5 mins. at 40°, then for 10 mins. at 80°, and finally for 10 mins. at 80-90°.

Potassium hydroxide-cellobiose recovered = 1.3 g.
Crystalline fraction obtained ..... = 0.5 g.
Syrupy fraction obtained ..... = 0.9 g.

Thus from 12 g. octa-acetyl cellobiose it was possible to obtain by repeated methylation of the recovered cellobiose-potassium hydroxide compound, 2.25 g. (19%) of a crystalline compound and 5 g. (41%) of syrup, with 1.3 g. (ca.16%) of the addition compound still remaining. This represents a recovery of ca. 75% of the starting material in three operations.

Examination of the Crystalline Fraction

Hepta-acetyl β-Methylcellobioside

The crude crystals were slightly reducing, but after 3 recrystallisations from alcohol a non-reducing product was obtained, and no sample of high reducing power could be isolated from the mother liquors during repeated attempts. The pure crystals had a m.p. of 178° and $[\alpha]_D^{20°} = -22°$ in chloroform (c,1.5). Zemplén (6) quotes m.p. 180°;and Hudson and Sayre (7) m.p. 187° and $[\alpha]_D^{20°} = -25.1°$ in chloroform.

Analysis:

Found: C, 50.0; H, 5.9; OMe, 4.5.

Calc. for $C_{27}H_{38}O_{18}$:

C, 49.9; H, 5.9; OMe, 4.9%.

All methoxyl analyses in this work were done by a method based on the micro-Zeisel method of Pregl, modified so that about 10 mg. of substance were used for a determination with compounds of methoxyl content 7-16%.

Examination of the Syrupy Fraction

Dimethyl Cellobiose Hexa-acetate, $C_{26}H_{38}O_{17}$

Analysis

Found: OMe, 8.1. Calc. for $C_{26}H_{38}O_{17}$;
OMe, 9.9%.

For deacetylation 0.1 g. required 9.63 c.c. N/10-NaOH
Calc. for hexa-acetate ..... 9.64 c.c.

$[\alpha]_D^{20^\circ} = +2^\circ$ (c, 0.5) in chloroform.

0.1882 g. required 0.6 c.c. N/10 iodine on treatment with alkaline hypiodite according to Bergmann and Machemer (8).

Since the reducing power of the syrup was negligible, it was thus a monomethyl methylcellobioside hexa-acetate. Experiments were then initiated to attempt to determine the position of the methyl group.

Hydrolysis of the Hexa-acetyl Methyl Methylcellobioside

The syrup was deacetylated by the method of Zemplén (5). 3.2 G. were dissolved in 10 c.c. of chloroform and a solution of sodium (0.25 g.) in methyl alcohol (10 c.c.) added. No sign of any solid addition compound was observed. The mixture was allowed to stand surrounded by a cold water bath for 1 hour, and was then extracted with water. The hydrolysis was carried out in 7% hydrochloric acid solution. Sufficient concentrated hydrochloric acid was added to the extract to bring it up to this strength, the volume being adjusted to make the concentration of sugar approximately 4%. The solution was heated on the water bath at 90°, hydrolysis being continued until the rotation of the solution became constant. This took about 5 hours.

Time (mins.)	0	65	125	220	300	380
$[\alpha]_D^{20^\circ}$	+3	13	27	37	50	50°

The solution was then neutralised with silver carbonate, filtered, and the solvent removed under reduced pressure at 50°.

On evaporation colloidal silver appeared from which the syrup was removed by extraction with methyl alcohol followed by removal of the solvent.

Analysis

Found: OMe, 5.0. Calc. for $C_6H_{12}O_6 + C_7H_{14}O_6$: OMe, 8.3%.

The low result was traced to the presence of some inorganic material.

Removal of Glucose

A solution of potassium hydroxide (1 g.) in absolute alcohol (15 c.c.) was added to the syrup dissolved in absolute alcohol (20 c.c.). After allowing to stand for 15 minutes the precipitated potassium hydroxide glucose compound was filtered, washed with absolute alcohol and dry ether and dried in a desiccator.

Yield 1.2 g. (Calc. 1.25 g.).

On dissolving this precipitated compound in water (10 c.c.), acidifying with acetic acid and treating with phenylhydrazine (1 g.) at 100° for 3 hours, glucosephenylosazone was isolated (0.3 g.). M.p. 206° [Found: OMe, nil.].

The solution gave no further precipitate on addition of alcoholic potassium hydroxide. A slight excess of glacial acetic acid was added and the solvent removed under diminished pressure to give a mixture of potassium acetate and a reducing syrup which was converted into the osazone.

Osazone Formation

The mixture of potassium acetate and syrup was dissolved in water (10 c.c.), and phenylhydrazine (1 g.) and glacial acetic acid (1 c.c.) added, and the solution heated at 90° for 30 minutes. An orange coloured osazone (0.5 g.) was obtained which was purified by recrystallisation from aqueous pyridine, m.p. 184°.

Analysis:

Found: OMe, 7.6; N, 14.75.

Calc. for Monomethyl glucosazone, $C_{19}H_{24}O_4N_4$:

OMe, 8.3; N, 15%.

In another similar preparation of osazone from dimethyl hexa-acetate syrup (2.0 g.) as starting material, a yield of 0.36 g. of the crude osazone

was obtained. After one recrystallisation this had a methoxyl content of 4.2% showing it to be contaminated with glucosazone. Further recrystallisation from aqueous pyridine raised the m.p. to 178-180°. Another portion recrystallised from aqueous alcohol melted finally at 180°. $[\alpha]_D^{20°} = -70°$ (initial) c, 0.3 in 50% alcohol-pyridine.

Analysis:

Found: C, 61.2; H, 6.6; OMe, 6.8; N, 14.7.

Calc. for monomethyl glucosazone $C_{19}H_{24}O_4N_4$,

C, 61.3; H, 6.45; OMe, 8.3; N, 15.0%.

The m.p. of 6-methyl glucosazone is quoted as 177° by Helferich and Becker (9) and 178-179° by Kuhn and Ziese (10). An authentic specimen was found to melt at 179°; $[\alpha]_D^{20°} = -68°$ (initial) c,0.3 in 50% alcohol-pyridine. 3-Methylglucosazone is quoted as melting at 178-179° by Freudenberg and Hixon (11) and also by Anderson, Charlton and Haworth (12). An authentic specimen was found to melt at 178°.

Mixed m.p. of Monomethyl and 6-Methyl Glucosazone 178 - 180°

" " " " " " 3-Methyl Glucosazone 164°

" " " " 6-Methyl and 3-Methyl Glucosazone 162 - 163°

From the fact that the melting point of the mono-methyl glucosazone was not depressed by admixture with 6-methyl glucosazone, the two substances were considered to be identical.

Action of Methyl Sulphate on Cellobiose

Pure crystalline cellobiose (1.4 g.), prepared by Zemplén's (5) method from the octa-acetate, was stirred at 70-75° for 45 minutes with excess of dry dimethyl sulphate. No reaction took place and the material was filtered and washed with acetone and ether to give unchanged cellobiose (1.3 g.).

Reaction between Methyl Iodide and the Cellobiose-Potassium Hydroxide

Cellobiose-potassium hydroxide compound was prepared from cellobiose octa-acetate (4 g.) in the manner previously described (p.26). The product, dried in a vacuum over phosphorus pentoxide, was boiled with methyl iodide (35 c.c.) for a period of 50 hours. A gummy solid was produced, from which the methyl iodide was removed under diminished pressure. The residue was acetylated by heating with acetic anhydride (5 c.c.) and pyridine (24 c.c.) at 80° for 5 minutes after which the mixture was allowed

to cool and stand for 24 hours. On pouring into water a precipitate (dry weight 1.8 g.) was obtained.

Examination of the Precipitate

On crystallisation from alcohol a crystalline solid was obtained, m.p. 192° and $[\alpha]_D^{20^\circ} = -8.8^\circ$ (c, 2) in chloroform. Found: OMe, nil. Further recrystallisation left these figures unchanged, and the substance was considered to be a mixture of α- and β-cellobiose octa-acetates.

Examination of the Solution

The solution was extracted with chloroform (1500 c.c.) and the extracts washed with dilute sulphuric acid to remove pyridine. Following further washing with sodium bicarbonate solution and water, the chloroform was removed under diminished pressure to yield a yellow syrup. This was treated with charcoal in alcoholic solution, the solution filtered and, after removal of the solvent, the syrup was heated at 70°/10 mm. for 24 hours. The syrup (1 g.) was reducing and had a methoxyl content of 2%.

On solution in alcohol and addition of 20% alcoholic potassium hydroxide (5 c.c.), potassium cel-

lobiosate (0.6 g.) was precipitated and removed by filtration. The alkaline filtrate was neutralised with glacial acetic acid, potassium acetate filtered off, and the solution concentrated under reduced pressure to a yellow syrup which was acetylated in the usual way with pyridine and acetic anhydride to yield a non-reducing syrup (0.2 g.).

Analysis

Found: OMe, 8.0%

Calc. for dimethyl cellobiose hexa-acetate, $C_{26}H_{38}O_{17}$:

OMe, 9.9%.

S_U_M_M_A_R_Y

1. A compound of cellobiose and potassium hydroxide containing two molecular proportions of the alkali to one of the disaccharide has been prepared.

2. Mild methylation of this compound with methyl sulphate followed by acetylation gave a monomethyl methylcellobioside hexa-acetate together with β-methylcellobioside hepta-acetate and unchanged cellobiose octa-acetate.

3. After deacetylation and hydrolysis of the monomethyl methylcellobioside hexa-acetate, 6-methyl glucose was obtained and identified as the crystalline phenylosazone.

4. From these results it is concluded that the potassium hydroxide residues are associated with the reducing group and with one of the primary alcoholic residues of the cellobiose. It is not clear, however, with which glucopyranose unit of the disaccharide this second potassium hydroxide residue is concerned.

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(4) Percival ---------- J.C.S., 1935, 650.

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(10) Kuhn and Ziese ------- Ber., 1926, 59, 2314.

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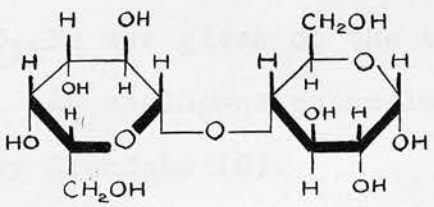
PART II

The Addition Compounds of Lactose and Potassium Hydroxide

The Addition Compounds of Lactose and Potassium Hydroxide

Attention was next transferred to the disaccharide lactose. This is similar to cellobiose in that it is a reducing sugar in which that unit of the molecule containing the free reducing group is a glucopyranose residue. The second unit of the molecule is not however derived from β-glucopyranose as in cellobiose but from β-galactopyranose:



Lactose

It has been noted that with cellobiose a difficulty in assigning a final structure to the methylated disaccharide obtained from the alkali compound was encountered, due to inability to distinguish between the two units of the degraded sugar. The choice of lactose was designed to overcome this difficulty since, if the lactose potassium hydroxide

compound were submitted to treatment similar to that undergone by the cellobiose alkali compound, the formulation of the methylated degradation products as derivatives of glucose or galactose would indicate the precise position of the substitution in the lactose molecule.

Little information on the alkali compounds of lactose is recorded in the literature. Hönig and Rosenfeld (1) reported the isolation of a sodium derivative of the sugar from sodium ethoxide and lactose in 98% alcohol at 50°, and to this the formula $C_{12}H_{21}O_{11}Na$ was given on the basis of analytical figures. An analogous potassium derivative was described by Brendeke (2).

Calcium lactosate, $C_{12}H_{22}O_{11},CaO$ was mentioned by Mackenzie and Quin (3), this being prepared from an aqueous solution of the components by precipitation with alcohol.

From these facts, amplified only by the determination by Hirsch and Schlags (4) of a dissociation constant for lactose as an acid, in aqueous solution of alkali, little can be gathered as to the constitution of the products formed.

The method employed in this thesis for investigating the relationship between lactose and potassium hydroxide was essentially that adopted for cellobiose, namely, mild methylation of the isolated compound. As before, the first step was to determine approximately the alkali combining capacity of the sugar, and this was done by the titration method previously described. From the results obtained (see table, p.55) it appeared that lactose possessed a greater affinity for potassium hydroxide than did cellobiose under similar conditions. In the range of alkali concentrations studied, the latter sugar took up a maximum amount of potassium hydroxide corresponding to the compound $C_{12}H_{22}O_{11}$,2KOH, whereas that taken up by lactose at the same concentration represented a compound intermediate between $C_{12}H_{22}O_{11}$,2KOH and $C_{12}H_{22}O_{11}$,3KOH. It was considered that a mixture of these two complexes was present, a view which the results of the methylation process served to confirm.

Lactose octa-acetate was suspended in absolute alcohol and an excess of absolute alcoholic potassium hydroxide added. The lactose alkali compound was

isolated, thoroughly dried, and subjected to a mild treatment with dry neutral dimethyl sulphate. Unchanged lactose was eliminated from the products by precipitation with potassium hydroxide in alcoholic solution, and acetylation of the remainder yielded a non-reducing syrupy mixture of the acetates of a monomethyl methyllactoside and a dimethyl methyllactoside, the combined yield being approximately 12.5% based on the weight of the initial lactose octa-acetate. No crystalline product could be obtained from this syrup, which was deacetylated and hydrolysed with dilute sulphuric acid. The resulting mixture of hexoses was isolated and examined with the object of identifying its methylated components.

Division of the mixture into its component parts proved troublesome and a complete separation was not attained. The first step consisted in removing unsubstituted sugars and this was effected by precipitation with potassium hydroxide in alcoholic solution. The addition of ether to the filtered solution precipitated a further sugar alkali compound, which on acetylation yielded a monomethyl hexose acetate. The sugars remaining in solution gave

an acetylated syrup which was derived from a mixture of monomethyl and dimethyl hexoses.

Additional purification was necessary before these products could be further examined, but this proved a matter of some difficulty, and whereas the monomethyl fraction could be obtained pure the methoxyl content of the higher methylated fraction could never quite be raised to that theoretically required for a dimethyl hexose triacetate.

Attempts to separate the acetate mixture by fractional precipitation from a chloroform solution with light petroleum were only partially successful, and fractional distillation in a high vacuum was found to be the most useful method to employ, although this was a matter of some difficulty owing to the high boiling points of the acetates. The last fraction to distil had the theoretical methoxyl content for a monomethyl hexose acetate but the purest specimen of the dimethyl derivative which could be obtained from the earlier fractions still contained from 20-25% of the monomethyl syrup.

The monomethyl and the dimethyl hexose acetate fractions were shown to consist entirely of galactose derivatives, since on appropriate treatment

2:3:4:6-tetramethyl galactose anilide was isolated from both with no trace of the corresponding glucose derivative. This showed that in the original lactose the substitution, with the exception of the methoxyl entering the reducing group, was taking place exclusively in the galactose half of the molecule.

The position of this substitution remained to be determined, and as in the case of cellobiose it had been seen that it occurred in a primary alcoholic residue the monomethyl galactose was examined for the presence of 6-methyl derivative. Repeated attempts were made to isolate 6-methyl galactose phenylhydrazone, but in every case the result was negative and it was decided that substitution had not occurred in position 6. The formation of a phenylosazone however provided more definite information since the monomethyl galactose gave on appropriate treatment a considerable yield of galactosazone (40% of the theoretical), the only conclusion to be drawn from this being that the methyl group had been eliminated by the phenylhydrazine residue and must therefore have been in position 2 in the galactose molecule (cf.

Robertson and Lamb (6)].

Under no conditions was it possible to isolate a dimethyl phenylosazone from the dimethyl fraction, the product being 4-methyl galactosazone, from which it was apparent that in the dimethyl galactose position 2 was again substituted. That the second methyl group was in position 4 as suggested by the isolation of the above product was confirmed by further experiments.

The change in specific rotation $[\alpha]_D^{18^\circ}$ $+49^\circ$ to $+33^\circ$ in 1 day observed during formation of the methylglycoside in methyl alcoholic hydrogen chloride (2%) at 18° was such that the existence of 2:3-dimethyl galactose was doubtful, Robertson and Lamb (6) having stated that on formation of the glycoside in this manner the specific rotation of a 2:3-dimethyl galactose solution fell from $[\alpha]_D^{15^\circ}$ $+38^\circ$ to $[\alpha]_D^{15^\circ}$ -24°, seven days being required to reach equilibrium at room temperature. Oxidation with nitric acid confirmed this view as attempts to isolate either d- or i-dimethoxy succinamide from the products failed, thus showing that the methyl residues were not in adjacent positions. This also eliminated 3:4-dimethyl galactose from consideration.

By cautious oxidation with bromine water a dimethyl lactone was produced and from the rate of decline of the positive rotation of its aqueous solution it was concluded that a δ-galactonolactone was present. As the conditions under which lactonisation had taken place were arranged to be favourable for the production of a γ-lactone this result was in conformity with the belief that the 4 position was blocked by a methyl residue.

Further confirmation was obtained by studying the course of glycoside formation with respect to the ring structure assumed by the product. By means of the method described by Levene, Raymond and Dillon (7) it was possible to follow the gradual conversion of the free sugar into galactofuranoside and galactopyranoside when treated with methyl alcoholic hydrogen chloride at room temperature, and the amount of each form comprising the product at any moment during the reaction was determined, although the results are not claimed to be strictly quantitative. With a specimen of 2:4-dimethyl galactose the product should have been entirely of pyranose structure, but it was found to contain a

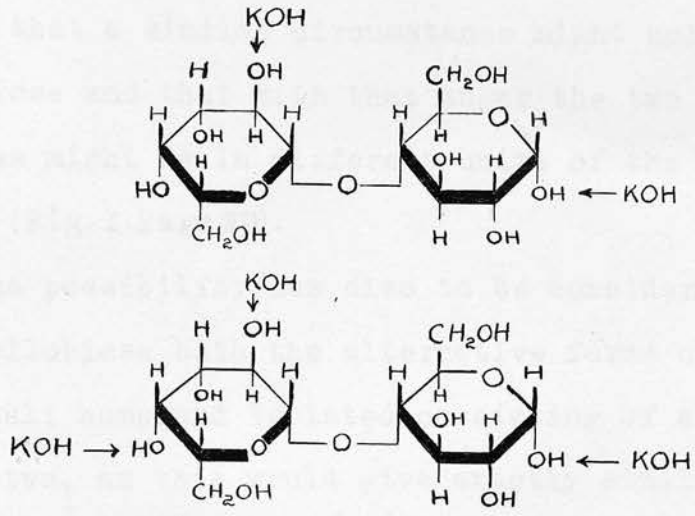
proportion of furanoside, the yield of which rose to a maximum of about 26% before finally decreasing. It has however been mentioned previously that isolation of the pure dimethyl galactose derivative was never accomplished, and, as the specimen in use was known to contain approximately 25% of 2-methyl galactose, the occurrence of furanoside was ascribed to this source.

Finally the fact that in the previously mentioned osazone formation no 6-methyl galactosazone was obtained ruled out the possibility of the presence of 2:6-dimethyl galactose.

Thus the structure of 2:4-dimethyl galactose was assigned to the dimethyl fraction under review.

It has been shown therefore that methylation of lactose potassium hydroxide compound, which appeared to be a mixture of $C_{12}H_{22}O_{11}$,2KOH and $C_{12}H_{22}O_{11}$,3KOH, gave rise to a monomethyl methyllactoside and a dimethyl methyllactoside. In the former the non-glycosidic methyl group was situated in position 2 in the galactopyranose unit of the molecule; in the latter the two methyl residues occupied positions 2 and 4, also in the galactose unit.

From these facts the deduction is made that lactose forms two compounds with potassium hydroxide, $C_{12}H_{22}O_{11}$,2KOH and $C_{12}H_{22}O_{11}$,3KOH. In both of these one alkali residue is situated at the reducing group, and the others are located at the positions indicated above as being occupied by methyl groups. These are indicated in the following formulae:



It is possible however that in addition to these compounds a compound of the formula $C_{12}H_{22}O_{11}$,KOH in which the reducing group only is concerned also exists as in the case of glucose and cellobiose, although a crystalline hepta-acetyl methyllactoside has never been isolated.

With cellobiose it had not been possible to decide between two alternative formulae for the potassium hydroxide compound, it not being known which primary alcoholic residue was reacting with the alkali. The fact that with lactose no further substitution took place in the glucose unit of the molecule after the reducing group had been satisfied suggested that a similar circumstance might hold for cellobiose and that with that sugar the two alkali residues might be in different units of the molecule. (Fig. I. Page 20).

The possibility has also to be considered that with cellobiose both the alternative forms occur, the alkali compound isolated consisting of a mixture of the two, as this would give exactly similar experimental results to those obtained. If it is the case however that there are two points within the cellobiose molecule, in addition to the reducing group, reactive towards potassium hydroxide, then a complex of the type $C_{12}H_{22}O_{11}$,3KOH might reasonably be expected, as with lactose, and no evidence for such a product was found.

E_X_P_E_R_I_M_E_N_T_A_L

Determination of the Alkali Combining Capacity of the Sugar

An approximate value for the alkali combining capacity of lactose was obtained by the titration method previously used for cellobiose.

A weighed portion of lactose monohydrate was dissolved in 75% alcohol and a measured volume of standard alcoholic potassium hydroxide was added. After standing for 10 minutes, the precipitated compound was removed by filtration through a Gooch crucible and an aliquot portion of the filtrate was titrated against standard acid using phenolphthalein as indicator.

The precipitate in the crucible was dried by suction and washed with the minimum quantity of absolute alcohol, the volume (5 c.c.) being kept constant for each determination. Dissolution in water and titration against acid then followed.

		I	II	III	IV
Conc^{n}. of Lactose %		0.72	0.94	1.00	1.01
Conc^{n}. of KOH	Initial Normality	0.221	0.427	0.747	0.774
	Final Normality	0.181	0.373	0.685	0.704
KOH in g. combined with 100 g. Lactose	Indirect Method	30.9	32.1	35.1	39.2
	Direct Method	28.1	26.0	34.2	41.6

For a compound of the type $C_{12}H_{22}O_{11}$,2KOH, 100 g. of lactose would require 32.8 g. potassium hydroxide, and for $C_{12}H_{22}O_{11}$,3KOH, 49 g. potassium hydroxide. Thus it would appear that in the higher concentrations examined some of the latter compound was present, the proportion of compound of alkali content lower than that required for $C_{12}H_{22}O_{11}$,3KOH increasing with diminishing concentration of potassium hydroxide. It was found impracticable to use very concentrated solutions of potassium hydroxide as this caused the decomposition of the sugar with the production of brown solutions.

Attempted Preparation of the Lactose Potassium Hydroxide Compound from Lactose

An attempt was made to prepare a compound directly from lactose and potassium hydroxide.

I. Lactose (10 g.) was dissolved in 70% aqueous alcohol (130 c.c.), and a solution of potassium hydroxide (10 g.) in alcohol (50 c.c.) added. No precipitate was obtained, as the product was immediately attacked by the water present with the formation of a yellow syrupy layer. Alcohol of the dilution used was necessary in order to dissolve a sufficient quantity of lactose.

II. Lactose (10 g.) was suspended in absolute alcohol (100 c.c.) and a solution of potassium hydroxide (10 g.) in absolute alcohol (60 c.c.) added. No compound formation was apparent and after 24 hours the unchanged crystalline lactose was recovered by filtration.

Preparation of Lactose Octa-acetate

This was prepared after the method of Hudson and Johnson (5). Acetic anhydride (400 c.c.) was heated to 100° in a flask of several litres capacity and anhydrous sodium acetate (25 g.) added. The temperature of the solution was raised almost to the boiling point and the addition of lactose monohydrate (100 g.) begun. After a little had been added a

vigorous reaction set in and the liquid commenced to boil. External heating was discontinued and the addition of sugar was continued until it was all in solution, the heat of reaction being sufficient to keep the liquid boiling during this period. Finally external heating was again applied and the reaction mixture boiled for 10 minutes, after which it was poured into a large volume of cold water. The insoluble viscous mass which was produced gradually solidified, the water being renewed from time to time. After standing for 3 days under water the product was broken up, filtered, washed with water, and dried in the air. Recrystallisation from hot 90% alcohol gave a white crystalline product.

Yield 95 g. (48% theoretical), m.p. 85°.

A Typical Preparation of the Lactose Potassium Hydroxide Compound

Lactose octa-acetate (35 g.) was suspended in absolute alcohol (200 c.c.) in a flask and a solution of potassium hydroxide (66 g.) in absolute alcohol (1000 c.c.) was added, the mixture being stirred mechanically for 2 hours. Owing to the fact that consid-

erable heat was developed during the reaction the flask was surrounded by cold water. After standing for a further 16 hours the precipitated compound was filtered quickly and washed with a minimum quantity of absolute alcohol. Sufficient alcohol had to be used to remove any adhering potassium hydroxide solution, otherwise the product was difficult to dry, but excessive washing must of necessity be avoided as tending to reduce the alkali content of the compound. After washing finally with dry ether the product was dried over phosphorus pentoxide in a vacuum desiccator.

Yield 20 g.

When dry the compound was a white, amorphous, deliquescent powder similar to the cellobiose derivative previously described.

A Typical Methylation of the Lactose Potassium Hydroxide Compound

To the finely powdered lactose potassium hydroxide compound obtained from lactose octa-acetate (35 g.), dry dimethyl sulphate (125 c.c.), which had

been neutralised with anhydrous potassium carbonate, was added. The temperature was maintained at 35-40° for 5 minutes, during which time the mixture was vigorously stirred in a closed flask. This was followed by raising the temperature to 70° for 10 minutes, after which it was allowed to cool. The brown viscid solid which remained, after decantation of the methyl sulphate, was washed thoroughly with acetone and then extracted with boiling methyl alcohol, after which the cold extract was allowed to stand for 2 hours to allow potassium methyl sulphate to crystallise. This was removed by filtration and a solution of potassium hydroxide (7 g.) in absolute alcohol (100 c.c.) was added to the filtrate. This precipitated unchanged lactose as the alkali compound, which was filtered off. The continued addition of alcoholic potassium hydroxide failed to bring about any further precipitation, but in order to bring about the complete removal of unchanged lactose ether was added. This caused a further precipitation of the sugar alkali compound, and addition was continued until the precipitate produced was no

longer reducing to Fehling's solution. The residual solution was acidified with galcial acetic acid and taken to dryness under reduced pressure. A mixture of syrup and potassium acetate was obtained and this was acetylated by heating at 95° for 1 hour with acetic anhydride (120 c.c.) and anhydrous sodium acetate (23 g.). The cold mixture was poured into water (500 c.c.), neutralised with sodium bicarbonate and extracted with chloroform (200 c.c.). The extracts were dried over anhydrous sodium sulphate and evaporated at 50° under diminished pressure to give a non-reducing syrup (4.4 g.) from which no crystals could be isolated.

Analysis

Found: OMe, 12.1.

Calc. for a dimethyl hexa-acetyl lactose, $C_{26}H_{38}O_{17}$,
OMe, 9.97%

Calc. for a trimethyl penta-acetyl lactose, $C_{25}H_{38}O_{16}$,
OMe, 15.66%.

Thus the syrup appeared to be a mixture of a monomethyl methyllactoside hexa-acetate and a dimethyl methyllactoside penta-acetate.

Hydrolysis of the Syrup

Deacetylation was carried out by Zemplén's (8) method. The syrup (4.4 g.) was dissolved in chloroform (10 c.c.) and a solution of sodium (0.5 g.) in methyl alcohol (10 c.c.) added. The mixture, surrounded by cold water, was allowed to stand for 1 hour, and was then extracted with water. To the aqueous extract (50 c.c.) of the de-acetylated sugar sufficient dilute sulphuric acid (30 c.c.) was added to make the resulting solution 1.5 normal. This was heated on the water bath at 95° till a constant rotation was attained.

Minutes	$[\alpha]_D^{20^\circ}$	Minutes	$[\alpha]_D^{20^\circ}$
0	+18°	395	+45.6°
150	+34.3	435	+47.4
290	+41.4	480	+48.0 (constant)

The solution was neutralised with barium carbonate, and, after removal of the insoluble barium salts by filtration, evaporated under reduced pressure at 50° to give a syrup contaminated with inorganic material.

Separation of the Products of Hydrolysis

The impure syrup was extracted with warm absolute alcohol (50 c.c.). To the extracts was added

an alcoholic solution of potassium hydroxide (2 g. in 40 c.c.), and a sugar alkali compound (I), which was immediately precipitated, was filtered rapidly, washed with alcohol and ether and dried in a vacuum over phosphorus pentoxide. Further addition of alcoholic potassium hydroxide to the filtrate had no effect, but the addition of dry ether (250 c.c.) immediately caused another precipitate (II) to be thrown out of solution, and this was likewise collected and dried. The residual solution was neutralised with acetic acid and evaporated under reduced pressure at 50° to yield a mixture of yellow syrup (III) and potassium acetate which was acetylated with acetic anhydride (35 c.c.) and anhydrous sodium acetate (7 g.) at 100° for 1 hour, a brown syrup being obtained after the usual working up. The dried sugar alkali compounds (I) and (II) were similarly acetylated with the production of syrups. Methoxyl determinations on these three acetylated fractions gave the following figures:

I. Yield 0.98 g. Found: OMe, 2.0%.

II. Yield 0.49 g. Found: OMe, 8.1%.

[Calc. for a monomethyl hexose tetra-acetate $C_{15}H_{22}O_{10}$: OMe, 8.56%]

III. Yield 0.83 g. Found: OMe, 15.5%

[Calc. for a dimethyl hexose triacetate $C_{14}H_{22}O_9$: OMe, 18.56%]

Thus an approximate fractionation of the mixture was achieved by this method

Fractional Distillation of the Acetylated Compound

A yellow syrup (5.6 g., OMe 11%) obtained as fraction III was introduced into a small distilling flask and distilled 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 temperature for a minimum period. The temperature of the bath surrounding the flask was raised to a maximum of 230°/.05 mm. pressure, and a clear yellow syrup (4.9 g.) distilled leaving a charred residue in the flask. Redistillation of the pro-

duct followed, during which several separate fractions were collected. Very little product came over below 163°, but at 165-168° (.04 mm.) a portion distilled steadily. When this ceased to come over the temperature had to be progressively raised to 180° (.05 mm.) to enable distillation to continue. At 182-183° the remainder of the syrup distilled almost completely. The following illustrates the separation effected.

	Temp.	Pressure	%OMe	Yield
1.	< 167°	.04 mm.	15.0	3.65 g.
2.	168-181	.05 mm.	12.2	0.75 g.
3.	182-183	.04 mm.	8.5	0.43 g.

The first of these fractions was submitted to a further distillation and that portion distilling from it below 162°/.05 mm. was collected.

Found: OMe, 16.7%, Yield, 0.4 g.

It was not possible to prepare by distillation any specimen with a greater methoxyl content and the yield of the fraction of this degree of purity was exceedingly small, amounting to 0.4 g. from 5.6 g. of original crude mixture.

Attempts were made to effect the separation of the crude acetate mixture by addition of light petroleum to a chloroform solution in the hope that the lower methoxyl containing fraction would prove more insoluble than the higher and be completely precipitated from the solution, leaving a mother liquor from which the dimethyl fraction could be isolated. While this method did effect some separation, it did not prove superior to distillation and was abandoned.

Complete Methylation of the Monomethyl Hexose Acetate

In order to determine whether this was derived from glucose or galactose it was converted into a fully methylated derivative. To a portion of the syrup (0.54 g., 8.5%OMe) dissolved in acetone (10 c.c.) and water (6.5 c.c.), dimethyl sulphate (10 c.c.) and 30% aqueous sodium hydroxide (25 c.c.) were added in small portions with constant stirring at intervals of 10 minutes. During the first three additions at 35° the amount of methyl sulphate was kept in excess of that of the alkali. The remainder of the reagents were added at 56° after which the solution was heated for 30 minutes at 75° before extracting

the reaction mixture with chloroform. The solvent was removed from the dried extracts to yield a syrup which was given two further methylations using Purdie's method. This consisted in refluxing at 50° with methyl iodide (10 c.c.) containing silver oxide (2g.) for 7 hours, followed by thorough extraction of the mixture with chloroform. After the second methylation the chloroform was removed from the extract to give a product which was distilled at 113°/.08 mm. to yield a clear mobile non-reducing syrup (0.26 g.).

The glucosidic methoxyl residue was removed by dissolving this syrup in 7% hydrochloric acid (10 c.c.) and heating at 100° for 2 hours. The solution was neutralised with barium carbonate, alcohol was added to precipitate barium salts which were filtered off, and the solution taken to dryness under reduced pressure. The residue was extracted three times with ether and from the filtered extracts a syrup (.19 g.) was obtained by evaporation. Two samples of this were innoculated with crystalline tetramethyl glucose and tetramethyl galactose respectively, but neither specimen showed any signs of crystallisation.

Preparation of the Tetramethyl Hexose Anilide

The anilide was prepared from the above sugar by boiling the syrup (0.19 g.) under reflux with aniline (0.6 g.) in alcohol (2 c.c.) as solvent for 3 hours. On cooling white needles separated, which were recrystallised twice from alcohol.

Yield 0.07 g.; m.p. 192-193°.

2:3:4:6-Tetramethyl galactose anilide gave m.p. 192°. A mixture of the preparation and a specimen of 2:3:4:6-tetramethyl galactose anilide melted at 191-192°.

No product corresponding to 2:3:4:6-tetramethyl glucose anilide, m.p. 138° could be isolated from the mother liquors. Therefore it was concluded that the sugar under investigation was a derivative of galactose.

The Attempted Isolation of 6-Methyl Galactose Phenylhydrazone

In order to test for the presence of 6-methyl galactose, attempts were made to prepare a phenylhydrazone after the method of Freudenberg and Smeykal (9). These authors stated that 6-methyl

galactose (1 g.), phenylhydrazine hydrochloride (2 g.) and crystalline sodium acetate (3 g.) dissolved in water (20 c.c.) gave colourless needles of the phenylhydrazone slowly in the cold, or after heating for 1 minute on the water bath.

A portion of syrup (0.4 g., OMe, 8.25%) was deacetylated by Zemplén's method to give the deacetylated sugar (0.2 g.), sodium acetate (0.8 g.) was added together with phenylhydrazine hydrochloride (0.4 g.) and the mixture was dissolved in water (4 c.c.). On standing for 12 hours there was no apparent change, and after being heated for 1 minute on the water bath and allowed to stand for a further period there was still no trace of any crystalline phenylhydrazone.

The experiment was repeated using conditions different from those described by Freudenberg and Smeykal. The method consisted in dissolving the sugar in a minimum amount of water and adding phenylhydrazine. When galactose was tested under these conditions a mass of crystalline galactose phenylhydrazone was produced overnight.

Another portion of the syrup (0.4 g.) was therefore deacetylated, and the aqueous extract of the sugar was evaporated under reduced pressure after neutralisation with acetic acid. The syrupy product, containing a little sodium acetate, was dissolved inwater (0.5 c.c.) and phenylhydrazine (0.5 c.c.) was added. The mixture was allowed to stand for several days at 0° without the appearance of any crystalline product, even after innoculation with crystals of authentic 6-methyl galactose phenylhydrazone. Rubbing with various solvents also failed to induce crystallisation.

It was therefore decided that 6-methyl galactose was not present in the specimens examined.

Osazone Formation

A specimen of the syrupy acetate (0.58 g. OMe, 6.5%) was deacetylated by Zemplén's method. The aqueous solution (20 c.c.), after addition of phenylhydrazine (1 g.), glacial acetic acid (2 c.c.), and sodium acetate (1 g.) was heated at 100° for $1\frac{1}{2}$ hours. To minimise the formation of tarry oxidation products a small amount of solid sodium bisul-

phite was added as recommended by Hamilton (10). After filtering off the osazone which had formed, heating was continued to obtain a second crop.

The unpurified first crop of osazone was found to have m.p. 170-175°. After several recrystallisations the melting point was raised to 186-188°. Yield 0.17 g. Found: OMe, nil. The melting point of this specimen was not depressed on admixture with galactosazone, hence it was assumed to be identical.

The second crop of osazone (0.08 g.) was similar to the purified first crop. M.p. 186-188°, OMe, nil. Thus total yield of galactosazone in the two crops = 0.25 g.

The isolation of galactose phenylosazone from a monomethyl galactose was taken as evidence that the methyl group was originally situated in the hydroxyl group at the 2-position in the galactose molecule. As however the material used did not appear to be a completely pure specimen of a monomethyl galactose tetra-acetate having only 6.5% methoxyl instead of the theoretical 8.56%, this galactosazone might possibly be considered to be

derived from the penta-acetyl galactose present as an impurity. For the following reasons however this could not be the case.

In the first place, if it were assumed that the lowering of the methoxyl content was caused by the presence of penta-acetyl galactose, then the amount of this substance present would not be sufficient to give the yield of galactosazone obtained above. From the methoxyl figures the maximum amount of penta-acetyl galactose which could be present in the 0.58 g. of acetate syrup was found to be 0.14 g., this being equivalent to 0.065 g. of galactose. A preparation of galactosazone from galactose was carried out under the same conditions as the osazone formation from the monomethyl sugar, and it was found that 1.85 g. of galactose would yield 2.25 g. of the osazone. Thus from 0.065 g. of galactose the amount of osazone obtainable would be 0.079 g. As the actual amount of galactosazone isolated from the sample of methylated sugar was 0.25 g., it could not therefore have been wholly derived from the penta-acetyl galactose.

That it was not even partly derived from this source was shown by repeated failure to confirm the

presence of any penta-acetyl galactose in the original syrup (OMe, 6.5%). Another portion of the syrup was deacetylated, the product dissolved in nitric acid (d,1.15) and the solution heated on the steam bath for 2-3 hours, finally being taken almost to dryness. On diluting with cold water and allowing to stand overnight, the solution remained quite clear, no crystallisation taking place. A similar experiment carried out on 0.1 g. of galactose gave a considerable yield of mucic acid. Thus the presence of 2-methyl galactose tetra-acetate in the original syrup was recognised.

Examination of the Dimethyl Hexose Acetate

The specimen was a colourless, viscous syrup, reducing to Fehling's solution. $[\alpha]_D^{20^\circ}$ +59° (c, 0.8 in chloroform). $n_D^{20^\circ}$ 1.4525.

Analysis

Found: C, 50.0; H, 6.4; OMe, 16.1.

Calc. for dimethyl hexose triacetate, $C_{14}H_{27}O_9$:

C, 50.3; H, 6.6; OMe, 18.5%.

Complete Methylation

In order to determine whether this was a glu-

cose or a galactose derivative, a portion (0.74 g.) was subjected to complete methylation as previously described for monomethyl galactose tetra-acetate. One methylation with methyl sulphate was employed, followed by two applications of methyl iodide and silver oxide, and the product, after purification by distillation at 113°/.08 mm. (yield 0.37 g.) was hydrolysed with 7% hydrochloric acid to remove the glycosidic methyl group. The resulting syrup (0.32 g.) which did not crystallise, was heated on the water bath for 3 hours with aniline (1 g.) and alcohol (3 c.c.). On cooling the solution deposited a mass of white needle-like crystals which were recrystallised from alcohol. Yield 0.2 g. m.p. 192-193°. Authentic specimen of 2:3:4:6-tetramethyl galactose gave m.p. 192°. Mixed m.p. 192°.

No trace of any product corresponding to 2:3:4:6-tetramethyl glucose anilide, m.p. 138°, could be found in the mother liquors, therefore it was concluded that the specimen under investigation was dimethyl triacetyl galactose.

Osazone Formation

I. A portion (0.27 g.) of the dimethyl triacetyl galactose was deacetylated by Zemplén's method. To

the solution of the deacetylated sugar in water (20 c.c.) were added phenylhydrazine (0.5 g.) in glacial acetic acid (2 c.c.), sodium acetate (1 g.) and a small amount of sodium bisulphite, and the whole heated on the water bath at 100°. Osazone formation proved to be very slow. On heating the solution for several hours, a dark oil gradually precipitated and this solidified on standing in the cold. After standing overnight, the dark lumps were broken up, filtered, washed with dilute acetic acid and dried to give a reddish-brown solid. Found: OMe, 7.5%
This was purified by solution in chloroform, filtration and precipitation therefrom by the addition of light petroleum. Found: OMe, 8.0%. The petroleum solution was evaporated to give a yellow glass.

Found: OMe, 8.1%.

Calc. for monomethyl galactosazone $C_{19}H_{24}O_4N_4$:

OMe, 8.3%.

Thus it appeared that from a dimethyl sugar a monomethyl osazone had been obtained. As this would establish one of the methyl groups in the 2 position, an exhaustive search for the presence of any dimethyl osazone was carried out.

A later crop of dark orange solid which came down from the osazone solution on further heating was found to have m.p. 150-154°. OMe, 7.2%. After 6 recrystallisations from aqueous alcohol a product of m.p. 186-187° and OMe, nil, was obtained. The melting point was not depressed by the addition of galactosazone, so the product was believed to be a specimen of this substance derived from 2-methyl galactose present as an impurity in the starting material.

The search for a dimethyl osazone was continued by extracting the original osazone solution with chloroform, washing the extract with dilute acetic acid, and evaporating. The glass so obtained gave OMe, 4.0%. Thus no trace of a dimethyl osazone could be found.

II. The above result was confirmed by another similar preparation, the amount of acetate syrup used being equivalent to 0.12 g. dimethyl galactose. Four crops of osazone were obtained.

The tarry material first formed was filtered, dissolved in chloroform and the solution washed with dilute acid, dried, and evaporated to give a glass.

Yield 0.02 g. Found: OMe, 6.6%.

2nd.crop	orange cryst. osazone	.023 g.	m.p.145-150°	OMe,7.1%
3rd. "	"	.016 g.	" 155-160	" 6.9
4th. "	"	.018 g.	" 160	" 4.7

The main solution was then extracted with chloroform, twice washed with hydrochloric acid and water, and the product on evaporation dissolved in ether from which it was then precipitated with light petroleum. Yield, .02 g. Found: OMe, 6.6%

The total yield of osazone was thus 0.1 g., and no trace of dimethyl osazone could be found in this product or in the various mother liquors.

Identification of the Osazone

The main portion of monomethyl osazone obtained gave the following figures: M.p. 145-150°. M.p. of a specimen of 4-methyl galactosazone 145°. Mixed m.p. with 4-methyl galactosazone, 146-148°.

Analysis:

Found: N, 14.9; OMe, 7.1.

Calc. for monomethyl galactosazone, $C_{19}H_{24}O_4N_4$:

N, 15.0; OMe, 8.3%.

No evidence for the presence of 3-methyl galactosazone (m.p. 176°) or 6-methyl galactosazone (m.p. 204°) was found.

On repeated recrystallisation of crops 3 and 4 an osazone of no methoxyl content, m.p. 186°, identical with galactosazone, was obtained.

From the above facts the acetate syrup under investigation appeared to be 2:4-dimethyl triacetyl galactose containing as impurity some 2-methyl tetra-acetyl galactose.

The Changes in Rotation during Glycoside Formation

A portion of the dimethyl galactose acetate syrup was deacetylated by Zemplén's method, and the aqueous extract of the free sugar taken to dryness under reduced pressure. The product was dissolved in methyl alcohol (10 c.c.) containing 1.9% of dry hydrogen chloride, the concentration of sugar in the solution being 0.94%. The solution was allowed to stand at room temperature and the change in rotation on the gradual formation of the glycoside observed.

Time hours	α	$[\alpha]_D^{18^\circ}$
0	+0.46°	+49°
19	0.32	34
43	0.31	33
67	0.31	33 (constant)

The equilibrium solution was non-reducing. These values differ from those given by Robertson and Lamb (6) for 2:3-dimethyl galactose, the rotation of which under the same conditions fell during seven days from $[\alpha]_D^{15^\circ}$ +38° to $[\alpha]_D^{15^\circ}$ -24°.

Glycoside Formation with the Dimethyl Galactose

A method described by Levene, Raymond and Dillon (7) was employed to follow the course of glycoside formation when the sugar was treated with methyl alcoholic hydrogen chloride at room temperature. Provided positions 4 and 5 in the molecule are both unsubstituted, a reducing sugar will give under such conditions a mixture of the furanoside and pyranoside forms of the glycoside, the less stable furanoside being initially produced and gradually converted to the more stable pyranoside structure. By the action of a dilute mineral acid the furanosides can be hydrolysed to the free sugar under conditions which leave the pyranosides unaffected. Determination of the amount of free reducing sugar present in solution before and after hydrolysis will therefore give by difference the amount of furanoside present at any

given moment. The difference between this and the total glycoside content of the solution before hydrolysis will give the amount of pyranoside. The free reducing sugar is estimated by the Hagedorn-Jensen method or by a micro modification of the Willstätter hypoiodite method, the latter actually being employed for the following experiments.

A portion of the deacetylated dimethyl syrup was dissolved in 0.5% methyl alcoholic hydrogen chloride, the concentration of sugar in the solution being approximately 9 mg. per c.c. Two samples of 0.5 c.c. each were withdrawn at intervals.

One sample was treated with 0.4N-sodium carbonate solution (0.5 c.c.) and water (3 c.c.), followed by the addition of 0.3N-sodium hydroxide (1 c.c.) and 0.03N-iodine (5 c.c.). After standing for 15 minutes the excess of iodine was liberated with 5N-sulphuric acid (0.2 c.c.) and titrated against 0.01N-sodium thiosulphate.

The second sample was heated for 10 minutes at 100° with 0.26N-hydrochloric acid (1 c.c.) and water (2 c.c.), after which the solution was quickly cooled and the acid neutralised with the calculated quantity

of 0.4N-sodium carbonate. 0.3N-Sodium hydroxide (1 c.c.) and 0.03N-iodine (5 c.c.) were added, and after 15 minutes the excess of iodine was determined as for the previous sample.

The difference between the figures obtained above and those obtained with blank experiments gave the values for the free sugar content of the glycosidic samples. Due to the pyranoside not being, as previously stated, entirely unaffected by the treatment with acid, the reducing values after such treatment had to be corrected for its partial hydrolysis. This was found to take place to the extent of 13%, a figure which was determined in a separate experiment by hydrolysing under the standard conditions a sample of the galactopyranoside prepared by refluxing the sugar with 5% methyl alcoholic hydrogen chloride for 7 hours followed by isolation in the usual way.

Table of Results

Time (hours)	0.01N-Thiosulphate, c.c.		Free sugar %			Composition of mixture %		
	Before hydrolysis.	After hydrolysis.	Before hydrolysis.	After hydrolysis.	After hydrolysis. corr.	Free sugar	Glycoside Furanoside	Glycoside Pyranoside
0	2.20	2.40	100	100	100	100	0	0
1	1.70	2.20	77.5	92	91	77.5	13.5	9
3	0.90	1.70	41	71	67	41	26	33
7.5	0.50	1.30	23	54	47	23	24	53
23.5	0	0.70	0	29	16	0	16	84

If the sugar in question was, as supposed, 2:4-dimethyl galactose, then no glycoside of furanoside configuration should have been formed. The results however showed that a maximum of 26% was obtained. This was explained by supposing it to be derived from the 2-methyl galactose which was known to be present in the samples used. The amount of this, calculated from the methoxyl content of the dimethyl acetate syrup, was ca. 25%, a figure agreeing with that above. Thus the results of this experiment did not invalidate the adoption of the 2:4-dimethyl galactose structure for the sugar under examination.

The Formation of a Dimethyl Lactone

Deacetylation of a specimen of the dimethyl galactose triacetate syrup (0.93 g.) was effected by dissolving it in methyl alcohol (20 c.c.) saturated with ammonia, and allowing the solution to stand for 24 hours. After this period the alcohol and ammonia were removed by evaporation under diminished pressure, and the acetamide was eliminated from the residual syrup by heating at 100°/.05 mm. This syrup was dissolved in water (20 c.c.) and oxidised with bromine (2.8 c.c.) for 22 hours at 25°, the bromine being removed by aeration, and the solution neutralised with silver carbonate. After filtration the silver salt of the acid was decomposed by the passage of hydrogen sulphide. The filtered solution of the free acid was taken to dryness, and the residual syrup heated at 100°/.05 mm. for 3 hours to bring about conversion to the lactone. (0.5 g.)

Analysis:

Found: OMe, 25.2%. Calc. for dimethyl galactonolactone, $C_8H_{14}O_6$; OMe, 30.1%.

Mutarotation of the Lactone

The lactone (.04 g.) was dissolved in water (7 c.c.) when the following mutarotation occurred:

Time	α	$[\alpha]_D^{20^\circ}$
5 mins.	0.28°	+49°
10 "	0.26	46
52 "	0.20	35
14 hours	0.14	24 (const.)

Titration showed that 75% of the lactone had been converted into the free acid at equilibrium.

Since this lactone was a galactose derivative, the positive rotation, by application of "Hudson's lactone rule", pointed to its formulation as a δ-lactone, and this was confirmed by the rate of decrease in rotation, which was comparatively rapid. These facts showed that, although the conditions of lactonisation were favourable for a γ-lactone, the 4th. hydroxyl group did not take part in ring formation, a fact which was in accordance with the belief that the specimen was for the most part a 2:4-dimethyl galactose derivative. It must be pointed out however that the rotation of the lactone was somewhat lower than would have been expected for a δ-galactono-

lactone. The probability is therefore that the 2-methyl galactose known to be present (ca. 20%) as an impurity was converted by the above treatment to the corresponding γ-lactone, which would have a negative rotation, thus diminishing the rotation observed.

Amide Formation

A portion of the above lactone was allowed to stand for 2 days with methyl alcoholic ammonia. After removing the solvent a yellow syrup was obtained which could not be induced to crystallise. The amide had $[\alpha]_D^{20^\circ} +34^\circ$, c, 0.5 in water.

Analysis:

Found: OMe, 22.6. Calc. for $C_8H_{17}O_6N$: OMe, 27.8%.

It is clear that this specimen is also contaminated with a monomethyl galactose derivative.

The Oxidation of Dimethyl Galactose

A portion of the acetate syrup (0.52 g.) was deacetylated by Zemplén's method and the aqueous solution of the sugar evaporated under reduced pressure. The product was dissolved in concentrated nitric acid, (5 c.c.; d, 1.42) and, after standing at room tempera-

ture for 2 hours, was heated at 85-90° for a further 6 hours. The excess nitric acid was then removed by distillation at 70° under reduced pressure for 48 hours with the addition of water in the usual manner. The solution was then taken to dryness and the product boiled under reflux with 6% methyl alcoholic hydrogen chloride (11 c.c.) for 7 hours. After neutralisation with silver carbonate and filtration, the product obtained by evaporation was distilled at 120°/.07 mm. (bath temp.). In addition to some crystals of methyl oxalate a liquid distillate (0.1 g.) was obtained. This was dissolved in methyl alcohol (5 c.c.), saturated with ammonia at 0°, and maintained at this temperature. After 4 days the solution was examined for both d- and i-dimethoxy succinamide with a negative result. This experiment was repeated and the absence of a dimethoxy succinamide was confirmed in the final product.

This was interpreted as showing that the two methyl groups present in the initial substance could not occupy adjacent positions in the molecule, thus eliminating the possibility of the presence of 3:4-dimethyl galactose.

S_U_M_M_A_R_Y

1. Titration experiments show that lactose combines with more potassium hydroxide than does cellobiose under the same conditions, the results indicating the presence of a mixture of $C_{12}H_{22}O_{11}.2KOH$ and $C_{12}H_{22}O_{11}.3KOH$.

2. Controlled methylation followed by acetylation results in the production of a syrup from which no crystalline hepta-acetyl methyllactosides can be isolated, but after hydrolysis and acetylation a tetra-acetyl monomethyl hexose and a triacetyl dimethyl hexose are obtained.

3. Complete methylation of both these products and the isolation of crystalline tetramethyl galactose anilide from each, together with the absence of any glucose derivatives, shows that the substitution has taken place in the galactopyranose portion of the lactose molecule.

4. From the monomethyl galactose no 6-methyl galactose phenylhydrazone can be obtained, showing the absence of substitution in position 6, but the isolation of galactosazone in 40% yield assigns position 2 to the methyl group.

5. From the dimethyl galactose no dimethyl osazone can be isolated, thus showing group 2 to be again substituted. The production of 4-methyl galactosazone suggests the remaining substitution to be in group 4. No trace of 6-methyl galactosazone is found.

6. The non-identity of the dimethyl galactose with the known 2:3-dimethyl galactose is demonstrated by the changes in specific rotation during glycoside formation. Titration experiments show that the major portion of the galactoside so produced exists as the galactopyranoside thus indicating substitution in position 4.

7. This conclusion is confirmed by oxidation giving a dimethyl δ-galactonolactone.

8. Vigorous oxidation with nitric acid followed by esterification and amide formation fails to reveal the presence of either d- or i-dimethoxy succinamide, hence the presence of adjacent methyl groups in position 2:3, or 3:4, appears to be excluded.

9. Evidence is therefore presented that 2-methyl galactose and 2:4-dimethyl galactose are derived from the addition compounds. It is therefore concluded that the potassium hydroxide residues probably occupy these positions in the galactopyranose unit of lactose.

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PART III

The Addition Compounds of Galactose and Potassium Hydroxide

The Potassium Hydroxide Compounds of Galactose

In the formation of the lactose potassium hydroxide compound, after the reducing group had been satisfied, the remaining alkali residues were all found to be situated in the galactopyranose unit, and it was considered that this idea might be extended to the case of cellobiose. This result might however have been due to galactose having a greater capacity for combining with alkali than glucose.

Very little investigation into the relations between galactose and alkalies appears to have been carried out, and reference to only two compounds has been discovered. Fudakowski (1) reported the production of a compound between galactose and barium hydroxide, in methyl alcoholic solution, to which the doubtful formula $(C_6H_{11}O_6)_4Ba_2.BaO$ was given, and Windaus (2) obtained, from an ammoniacal zinc hydroxide solution of the sugar, the complex $(C_6H_{12}O_6)_2.3NH_3.Zn(OH)_2.2$ or $3H_2O$. It was decided therefore to examine the relationship between galactose and potassium hydroxide by the methylation method previously adopted.

On account of the very small solubility of galactose in absolute alcohol and also since the presence of a trace of water led to the formation of syrupy products it was necessary to make all the preparations from the acetylated sugar.

The titration method for determining the amount of combined alkali had also to be conducted on the acetate, and in consequence the method had to be modified somewhat. The results (Table p. 92) showed that the compound formed was probably of the type $C_6H_{12}O_6$,KOH, and the methylation process confirmed this view. After the usual treatment with neutral dimethyl sulphate, followed by removal of much unchanged galactose by treatment with potassium hydroxide, acetylation gave a yield of 25% of a non-reducing syrup which was evidently a mixture of the α- and β-forms of methylgalactoside tetra-acetate.

From these facts it was therefore concluded that galactose formed a compound with potassium hydroxide of the type $C_6H_{12}O_6$,KOH in which the alkali residue, as in the case of glucose, was situated at the reducing group.

E_X_P_E_R_I_M_E_N_T_A_L

Galactose Penta-acetate

Galactose penta-acetate was prepared in the usual way and on recrystallisation from hot alcohol had m.p. 142°.

Alkali Combining Capacity of Galactose

A series of titrations was carried out on the production of the galactose potassium hydroxide compound from galactose penta-acetate and potassium hydroxide. An alteration in the usual method had to be made since in this case there were two processes taking place, namely deacetylation and compound formation, both of which were responsible for removing alkali from solution. Since the amount of potassium hydroxide required for deacetylation was not that theoretically necessary for 5 acetyl groups, owing to deacetylation by alkali in alcoholic solution being a catalytic process (cf. Zemplén (4)), the normal method was modified as follows.

To each of two similar suspensions of galactose penta-acetate (0.2 g.) in absolute alcohol (5 c.c.), a definite quantity of standard alcoholic potassium hydroxide was added. One sample was then treated in

the usual manner, i.e. filtration and titration of the filtrate, and from the decrease in alkalinity the total amount of potassium hydroxide removed from solution was determined. By titrating the second sample without previous filtration the quantity of alkali required for deacetylation was determined. By difference, therefore, the potassium hydroxide engaged in compound formation was ascertained. A second value was obtained by direct titration of the compound as before.

Results

		I	II	III
Conc.^{n} of KOH	Initial Normality	0.27	0.41	0.61
	Final Normality	0.07	0.18	0.41
KOH in g. required to deacetylate 100 g. penta-acetate		68.5	72.0	69.0
KOH in g. combined with 100 g. galactose	Indirect Method	18.0	32.2	27.0
	Direct Method	25.3	26.1	27.5

For a compound $C_6H_{12}O_6$,KOH, 100 g. of galactose require to combine with 31.2 g. of potassium hydroxide

Typical Preparation of the Galactose Potassium Hydroxide Compound

Galactose penta-acetate (18 g.) was suspended in absolute alcohol (200 c.c.) and a solution of potassium hydroxide (45 g.) in absolute alcohol (1000 c.c.) was added gradually while the mixture was mechanically stirred in a closed flask. After being allowed to stand for 18 hours, the sugar alkali compound was filtered from the mixture, washed rapidly with a minimum quantity of absolute alcohol and dry ether and dried over phosphorus pentoxide in a vacuum. The product (10 g.) was a white deliquescent powder which was more sensitive to moisture than the compounds previously described.

A Typical Methylation of the Galactose Potassium Hydroxide Compound

The compound (10 g.) obtained in the last preparation was powdered and treated with neutral dimethyl sulphate (75 c.c.). The mixture was vigorously stirred in a closed flask for 10 minutes at 35-40°, followed by a further 10 minutes at 70°, and

then allowed to cool. The excess of methyl sulphate was poured off and the solid was dissolved in a minimum amount of boiling methyl alcohol. The solution was allowed to stand for 2 hours to deposit potassium methyl sulphate, which was filtered, and to the filtrate was added, in order to precipitate unchanged galactose as the alkali compound, a solution of potassium hydroxide (8 g.) in absolute alcohol (100 c.c.). This was removed and ether (1200 c.c.) added, which brought down a further quantity. The refiltered solution was then acidified with glacial acetic acid, evaporated under reduced pressure, and the mixture of syrup and potassium acetate acetylated with acetic anhydride (120 c.c.) and anhydrous sodium acetate (20 g.) at 100° for 1 hour, the solution then being poured into cold water, neutralised with sodium bicarbonate and extracted with chloroform. The extracts were dried over sodium sulphate and evaporated under reduced pressure to yield a non-reducing syrup (4.5 g.)

Analysis:

Found: OMe, 8.1. Calc. for tetra-acetyl methylgalactoside $C_{15}H_{22}O_{10}$:

OMe, 8.6%.

$[\alpha]_D^{18^\circ}$ + 21.7° c, 2.3 in chloroform.

S_U_M_M_A_R_Y

1. By titration experiments it is found that galactose appears to form a compound of the type $C_6H_{12}O_6$.KOH with potassium hydroxide

2. This formulation is supported by methylation with dry methyl sulphate, followed by acetylation with the formation of a mixture of tetra-acetyl α- and β-methyl galactosides.

3. From this result it is concluded that the reducing group of the galactose is associated with the potassium hydroxide.

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PART IV

The Addition Compounds of α-Methylgalactoside and Potassium Hydroxide

The Potassium Hydroxide Compounds of α-Methylgalactoside

In the preceding parts of this thesis the compounds formed between cellobiose, lactose and galactose have been examined, and in each case it has been found that, while other hydroxyl groups in the molecule may or may not be concerned, the reducing group of the sugar is always an active participant in the union with alkali. Bearing this fact in mind it was decided to consider the case of a sugar derivative containing no free reducing group. The sugar which at once rises to mind is sucrose and the fact that this disaccharide forms many compounds with alkalies is well known (1).

Examination of the literature would suggest however that the great reactivity of sucrose in this direction does not extend to other sugars in which the reducing group is blocked. Although raffinose shows as would be expected similar tendencies to those shown by sucrose, yet in the case of the simplest examples, i.e. the methylglycosides, no such compounds with alkalies are recorded. Indeed the non-existence of such compounds is reported by some workers. Mackenzie and Quin (2) were unable to find

any evidence of compound formation between calcium hydroxide and the methyl-glucosides, -fructosides, -maltosides, and -lactosides, and Percival (3) stated that α- and β-methylglucosides appeared to form no addition compounds with potassium hydroxide.

It was however decided to investigate α-methyl-galactoside from this standpoint since in lactose galactose appeared to take up two molecular proportions of potassium hydroxide. It was found that α-methylgalactoside in alcoholic solution gave no precipitate with alcoholic potassium hydroxide solution. On the addition of ether however a white powder was precipitated which appeared on examination to be an addition compound of α-methylgalactoside and potassium hydroxide. Ether had not been used in the attempts to prepare addition compounds of the α- and β-methylglucosides and potassium hydroxide (3).

Owing to this solubility in alcohol the alkali combining capacity of the galactoside could not be determined by the method usually employed and the one of direct analysis of the isolated compound had to be used. No great accuracy is claimed for this method, but the figures, ranging from 24 g. to 34 g. of potassium hydroxide combined with 100 g. α-methyl-

galactoside (see Table p.101), seemed to indicate that a compound of the type $C_7H_{14}O_6$,KOH was possible.

The product was subjected to the action of neutral dimethyl sulphate in the usual manner. Unchanged methylgalactoside was eliminated as far as possible by precipitation with alcoholic potassium hydroxide and ether, and the residual product was acetylated to give a syrupy mixture of acetates. From this mixture a sample of a monomethyl triacetyl methylgalactoside was obtained almost analytically pure by fractional distillation in a high vacuum, although a sharp separation from tetra-acetyl α-methylgalactoside was difficult.

It was shown that from the monomethyl triacetyl methylgalactoside, after deacetylation and hydrolysis, a yield of galactose phenylosazone (15-20%) could be obtained, and as this could not be derived to any great extent from any tetra-acetyl methylgalactoside present as impurity in the specimen, it was concluded that the methyl group had been eliminated on formation of the osazone and that it must therefore have been in position 2 in the galactose molecule.

On the other hand, the crude osazone had, before purification,a methoxyl content of ca. 2%, but at-

tempts to isolate and identify a specimen of a monomethyl osazone were not successful. From the analyses of the α-methylgalactoside potassium hydroxide compound it would seem likely that only one position is concerned, at least in the range of alkali concentrations studied, but further investigation will be necessary to decide whether substitution in additional groups can also occur.

From these results it is concluded that α-methylgalactoside forms a compound with potassium hydroxide of the type $C_7H_{14}O_6$,KOH in which the alkali residue is located at position 2 in the galactoside molecule.

The results of these experiments with α-methylgalactoside as well as those with lactose suggest that galactose in glycosidic union can attract more potassium hydroxide residues than in the case of galactose itself, but this may be connected with questions of solubility. This would appear to destroy the evidence on which the formulation of the potassium cellobiose compound rests (Fig.I.p.20), but since galactose combined in lactose takes up two potassium hydroxide residues whereas in α-methylgalactoside only one was detected there is strong reason to suppose that the non-reducing portion of a disaccharide is in some way more able to combine with alkali than the unit carrying the reducing group.

E_X_P_E_R_I_M_E_N_T_A_L

α-Methylgalactoside

α-Methylgalactoside was prepared according to the method described by Patterson and Robertson (4) for α-methylglucoside. The product was recrystallised from absolute alcohol and gave m.p. 109°, $[\alpha]_D^{20^\circ}$ +176°, c,5 in water.

A Typical Preparation of α-Methylgalactoside Potassium Hydroxide Compound

α-Methylgalactoside (10 g.) was dissolved in warm absolute alcohol (50 c.c.) and to the cold solution was added a solution of potassium hydroxide (12.5 g.) in absolute alcohol (175 c.c.). No apparent reaction took place, the mixture remaining quite clear, but on the addition of dry ether (400 c.c.), a precipitate was produced. This was rapidly filtered, washed once with ether, and dried in a vacuum over phosphorus pentoxide. The product was a white deliquescent solid, Yield 9 g.

The Alkali Combining Capacity of α-Methylgalactoside

Several preparations of the compound were carried out in the manner described above, the strength

of the alcoholic potassium hydroxide however being varied for the different preparations. A portion (ca. 0.2 g.) of the dried compound was weighed out in a closed vessel, dissolved in water, and titrated against standard hydrochloric acid to determine the alkali content. From this the amount of potassium hydroxide combining with 100 g. of the methylgalactoside was calculated.

	I	II	III	IV	V	VI
Conc.^{n} of α-Methyl-galactoside %	4.3	4.4	4.8	5.0	4.3	4.3
Initial normality of KOH in the original galactoside-alkali solution	0.78	0.99	1.09	1.33	1.94	1.94
KOH in g. combined with 100 g. α-methylgalactoside	28.7	28.9	33.6	24.7	34.3	26.5

It must be stated however that no great reliance can be placed on these figures, owing to the uncertain factor introduced by the necessary washing of the compound, which, if insufficient, would fail to remove all the adhering alkali solution, and, if excessive, would tend to reduce the combined alkali content of the compound. Although for this reason

the values obtained do not show any great regularity, they do nevertheless seem to indicate the possibility that the compound formed is of the type $C_7H_{14}O_6$,KOH, as, for such a compound,100 g. of the galactoside would require to combine with 28.9 g. of potassium hydroxide.

Typical Methylation of the α-Methylgalactoside Potassium Hydroxide Compound

The compound (12 g.) with dry neutral dimethyl sulphate (45 c.c.) was vigorously stirred at 35-40° for 5 minutes and then at 70° for 10 minutes, after which the mixture was allowed to cool. The excess methyl sulphate was removed and the resulting sticky solid was washed with acetone, dissolved in a minimum amount of boiling methyl alcohol, and the potassium methyl sulphate, which crystallised on cooling, was filtered off after 1 hour. A solution of potassium hydroxide (6 g.) in absolute alcohol (75 c.c.) was added, followed by ether (350 c.c.), and the alkali compound of the unchanged galactoside thus produced (11 g.) was removed by filtration. The filtrate was neutralised with glacial acetic acid and evapor-

ated under reduced pressure to give a mixture of syrup and potassium acetate which was acetylated by heating with acetic anhydride (40 c.c.) and anhydrous sodium acetate (5 g.) at 100° for 1 hour. After working up in the usual manner a syrup (1.4 g.) was obtained.

Analysis:

Found: OMe, 11.0. Calc. for a monomethyl triacetyl methylgalactoside, $C_{14}H_{22}O_9$:

OMe, 18.6. Calc. for tetra-acetyl methylgalactoside, $C_{15}H_{22}O_{10}$:

OMe, 8.6%.

The syrup appeared to be a mixture of these two substances, the precipitation with alcoholic potassium hydroxide and ether having failed to remove completely from the products of methylation the unchanged galactoside on account of the solubility of the addition compound.

Isolation of a Monomethyl Methylgalactoside

The syrup was distilled in a high vacuum but as in the case of the mixture of 2-methyl galactose tetra-acetate and 2:4-dimethyl galactose triacetate

obtained during the investigation of lactose a sharp separation was not obtained. A first fraction (0.17 g.) was collected at 160° - 165°/0.10 mm.

Analysis:

Found: OMe, 17.7. Calc. for $C_{14}H_{22}O_9$: OMe, 18.6%.

This proved to be the purest specimen obtainable, the next fraction collected at 165-173°/.08 mm. having a methoxyl content of 13.3%.

Osazone Formation

The monomethyl triacetyl methylgalactoside (0.33 g., OMe, 17.7%) was deacetylated by Zemplén's method, and the glycosidic methyl residue was removed by hydrolysing the product in 2% solution with 4% sulphuric acid at 100° for 4½ hours, the acid then being neutralised with barium carbonate and the solution filtered.

Phenylhydrazine (0.4 c.c.), dissolved in glacial acetic acid (0.9 c.c.), and a small quantity of sodium bisulphite were added to the filtrate, and the solution was heated at 100° for 2 hours. The yellow phenylosazone which separated on cooling was filtered, washed with dilute acetic acid and water,

and dried in a vacuum over phosphorus pentoxide. The crude product (0.07 g.) (20%) m.p. 148-152° gave OMe, ca. 2%. By repeated recrystallisation from hot aqueous alcohol the melting point was raised to 184-186° (OMe, negligible) and a mixed melting point of the pale yellow product with galactosazone showed a depression of 2° and hence was considered to be identical.

From the combined mother liquors a very small quantity of a dark orange solid, m.p. 148°, was obtained by the further addition of water, but the amount available was insufficient for further examination.

The amount of osazone obtained (0.07 g. OMe, 2%) corresponds to at least 0.05 g. of galactosazone assuming the impurity to be a monomethyl galactosazone. This corresponds to a yield of 15% of the starting material. If to account for the slightly low methoxyl value (17.7%) one assumes the impurity to be entirely α-methylgalactose tetra-acetate, 0.017 g., or less than 5%, of galactosazone would be expected. Furthermore oxidation with nitric acid failed to produce mucic acid.

A sample of the above monomethyl methylgalactoside acetate (0.3 g.) was deacetylated and treated with nitric acid (d.1.15) at 100°, a control experiment on α-methylgalactoside being carried out at the same time. No mucic acid could be found in the sample tested although the control experiment gave a positive result.

S_U_M_M_A_R_Y

1. By the addition of ether to an alcoholic solution of α-methylgalactoside containing potassium hydroxide a complex has been isolated.

2. Direct analysis showed that the probable composition of this could be represented by $C_7H_{14}O_6.KOH$.

3. Controlled methylation followed by acetylation resulted in the isolation of a triacetyl methyl methylgalactoside.

4. On treatment of the monomethyl galactose derived from this, galactosazone in a yield of 20% was isolated, thus assigning position 2 to the methyl group.

5. From the above results it is concluded that the potassium hydroxide residue is associated with the hydroxyl group in position 2 in the α-methylgalactoside.

Bibliography

1. Percival ----------J.C.S., 1935, 648.

2. Mackenzie and Quin ---------J.C.S., 1929, 951.

3. Percival ----------J.C.S., 1934, 1160.

4. Patterson and Robertson ----J.C.S., 1929, 300.

The Structure of the Sugar Alkali Compounds

The Structure of the Sugar Alkali Compounds

From a survey of the facts set out in the introduction to this thesis it is clear that in the formation of compounds of the sugars with metallic oxides and hydroxides we are dealing with a definite chemical reaction and it therefore remains to discuss the probable nature of the linkages involved.

The question then arises as to whether we are dealing with substitution compounds comparable to those of the alcohols, e.g. sodium ethoxide, or addition compounds in which the inorganic residue is co-ordinated to some reactive point within the sugar molecule.

As previously indicated the earlier workers generally formulated their products on the first supposition, potassium glucosate being written as $C_6H_{11}O_6K$. These formulae were based mainly on direct analyses of the compounds, but as it has been seen that such sugar potassium compounds are rather unstable and tend to decompose when attempts are made to obtain them in a pure state free from adhering mother liquor, it does not seem likely that distinction could be made, by direct analysis, between

the two possibilities $C_6H_{11}O_6K$ and $C_6H_{12}O_6,KOH$.

Doubt soon came to be cast on the alcoholate formula due to the failure to apply such compounds to synthetic experiments. If the metal had replaced a hydrogen atom in the sugar as in the alcoholates then it should have been easily replaced by a group such as the methyl group with the formation in good yield of a methyl ether. Mackenzie and Quin (1) attempted to methylate a lime sucrose compound in aqueous solution with methyl sulphate but were unable to isolate any identifiable methylated products.

In this connection it is notable that Muskat (2) has isolated sugar potassium compounds from which an almost theoretical yield of a methylated derivative could be obtained by the action of methyl iodide. These compounds were formed by the addition of metallic potassium to solutions of sugars or their derivatives in liquid ammonia and it would seem that in this case compounds of the alcoholate type were formed.

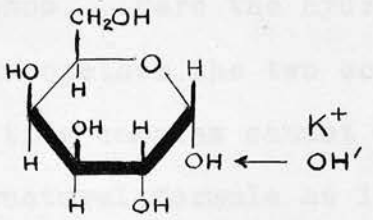
In the present work by the action of neutral methyl sulphate on the sugar alkali compounds under strictly anhydrous conditions it was possible to

replace by a methyl group each alkali residue previously shown to be present by analysis, the amount of unchanged compound however being very considerable.

Thus owing to the exceedingly poor conversion to methylated sugars achieved when working with the compounds prepared by the use of potassium hydroxide, it was not considered probable that these compounds could be of the same type as those described by Muskat. Furthermore the isolation of compounds such as $C_6H_{12}O_6, NaOC_2H_5$ from glucose and sodium ethoxide in the complete absence of moisture appeared to support the addition compound theory (3). The union of the potassium hydroxide and sugar however appeared to be feeble and it was considered very likely to be due to some form of co-ordination.

The formation of a co-ordinate link requires the presence of 2 atoms, one with a pair of unshared valence electrons and another able to accept such a pair. Here we have this condition fulfilled, the typical associating group, hydroxyl, with both a strong donor and acceptor being present in both the sugar

and the potassium hydroxide. Considering the case of galactose, it has been seen that the inorganic residue is attached to the reducing group. The fact that with a glycosidic galactose the KOH ceases to be associated with the blocked reducing group and becomes attracted instead to the hydroxyl in position 2 would seem to show that the oxygen of the sugar hydroxyl group does not act as a donor of electrons but that the hydrogen of this group acts as an acceptor of electrons from the oxygen atom of the alkali hydroxide.

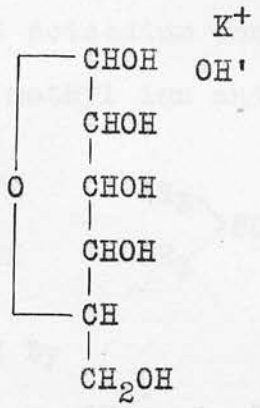


This behaviour of potassium hydroxide is in accordance with the formation by alkali hydroxides of stable monohydrates of which the occurrence is attributed to hydration of the negative hydroxyl ion, HOH ← OH', the oxygen atom of which acts as a powerful donor of electrons [Sidgwick (4)].

It is of interest to note that lactose in common with many galactose derivatives forms a stable

monohydrate more readily than glucose and its derivatives. The fact that lactose and α-methylgalactoside appear to be able to accommodate more potassium hydroxide residues than cellobiose and the corresponding glucose compounds may be connected with this greater 'residual affinity'.

In formulating these co-ordination compounds the linking hydrogen atom is shown as taking up a maximum of four valence electrons, but in the light of modern views on the co-ordination of hydrogen this is doubtful and an alternative system is provided by the theory of resonance. Here the hydrogen atom still serves to link together the two combining compounds, but the resulting complex cannot be represented by one static structural formula as it possesses simultaneously the properties of two different formulations in which the hydrogen is covalently attached to first one and then the other of the two constituents, in this case the alkali and the sugar residue. We have thus the two resonating forms:



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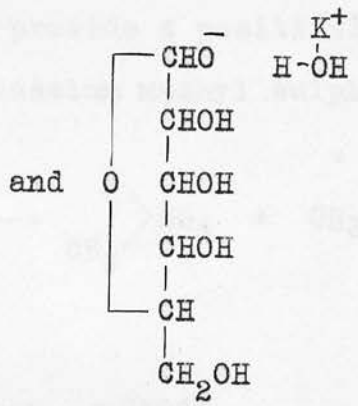
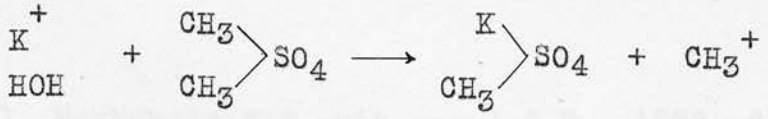


II

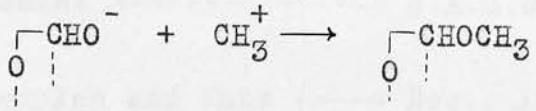
Although written as above for the sake of clarity it is not implied that the hydrogen atom oscillates from one position to the other but that the orbits of the valence electrons by which it is bound oscillate. To enable this to happen the alkali and sugar residues must be even closer to each other than would be necessary if they were bound by a normal covalent bond, thus producing a moderately stable complex. These considerations could be equally well applied to hydroxyl groups in the sugar molecule other than the reducing group.

Formula II above might help to explain the mechanism of the reaction with methyl sulphate involving the entry of an alkyl group into the sugar molecule provided the methyl sulphate reacts first with the

hydrated potassium ion to provide a positively charged methyl ion and potassium methyl sulphate thus



followed by



It must be pointed out however that Fajans and Born (5) regard the hydration of an anion to be a physical cohesion of dipoles rather than a 'co-ordination' process. At this stage therefore it does not seem desirable to define the union between the sugars and alkali hydroxides other than by some type of loose combination probably best represented as a dotted line: CHOH.....KOH in the manner of the original residual valencies of Werner.

Bibliography

1. Mackenzie and Quin --- J.C.S., 1929, 951.

2. Muskat ---------- J.A.C.S., 1934,56,693; 2449.

3. Zemplén and Kunz ----- Ber., 1923,56, 1705.

4. Sidgwick ---------- "Electronic Theory of Valency", Oxford,1927.

5. Fajans and Born ------ Ann.Reports,1934,p.89.

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This is to certify that Mr GEOFFREY G. RITCHIE, a candidate for the degree of Ph.D., successfully sustained an oral examination by a Committee of the Department on the subject of his thesis on 19 March, 1936.

Chairman of Committee.

8 May, 1936.