251. *Addition Compounds of the Carbohydrates. Part I. Potassium Hydroxide–Glucose and Related Compounds.*

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The fact that sugars and polysaccharides appear to form compounds with the hydroxides of the alkali and alkaline-earth metals has been known for many years, but the precise nature of such compounds is still in doubt, research having been directed rather to the elucidation of stoicheiometric relationships between the organic and inorganic constituents than to the problems of structure.

Several workers have attacked the problem from a physicochemical standpoint, regarding the sugars as weak acids. Madsen (*Z. physikal. Chem.*, 1901, **86**, 290) calculated the heat of neutralisation of glucose and sodium hydroxide to be 5340 cals. from determinations of the rate of hydrolysis of ethyl acetate by sodium hydroxide in the presence of glucose, assumed to be a weak monobasic acid. Hirsch and Schlags (*Z. physikal. Chem.*, *A*, 1929, **141**, 387), however, from conductivity measurements, conclude that glucose behaves as a feeble dibasic acid, $K_1 = 7{\cdot}82 \times 10^{-13}$, $K_2 = 1{\cdot}54 \times 10^{-14}$ at 25°.

That compounds appear to be formed between glucose and alkali even in aqueous solution was pointed out by Groot (*Biochem. Z.*, 1924, **146**, 72; 1927, **180**, 340), who observed that the maximum depression of the specific rotation of a glucose solution in the presence of potassium or sodium hydroxide occurred when the constituents were present in approximately molecular proportion. From measurements of the rate of decline of rotation at different concentrations, he concluded that the dissociation constant of glucose as an acid was $K = 8{\cdot}6 \times 10^{-13}$ at 25°, 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; but the possibility of the formation of other sugars during these transformations lessens the force of the argument. The cause of the apparent acidity and the point at which it arises in the molecule cannot be decided by experiments along these lines, although Michaelis and Rona (*Biochem. Z.*, 1913, **49**, 232), 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 $-CH(OH){:}C(OH)-$. This, however, is open to the objection that such an explanation could only apply to the reducing sugars, and the many compounds which sucrose forms with alkali and alkaline-earth hydroxides cannot therefore be explained on this basis. Such physicochemical results simply serve to show that the sugars examined remove alkali or hydroxyl ions from the solution.

It was decided therefore to institute investigations with the object of deciding at which points in the molecule the inorganic constituents are attached, and the nature of the linkages involved. The isolation of about 80 authenticated compounds from various

carbohydrates and alkalis which appear to contain the metallic hydroxide (or oxide) and sugar in stoicheiometric proportion (see, *e.g.*, Mackenzie and Quin, J., 1929, 951), seems to preclude the possibility that the phenomenon is due to adsorption or to a fortuitous precipitation of the metallic constituent along with the sugar residue. In particular, when both the reacting hydroxide and the sugar are soluble in the reaction medium, simple mixing affording a precipitate of the compound, it would seem to be clearly a case of chemical reaction.

It remains to decide therefore whether the " saccharates " are substitution compounds comparable with sodium ethoxide, or co-ordination compounds in which the inorganic residues are attached to the hydroxyl groups of the sugar molecule by covalent links. For this purpose potassium glucosate was selected for a preliminary examination.

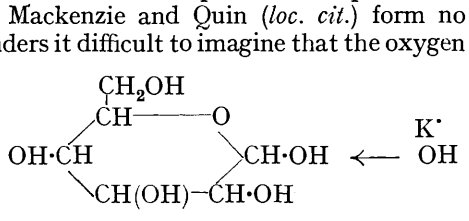
It was assumed by Marchlewski (*Ber.*, 1893, **26**, 2928) that the alkali metal was attached to the reducing group, but this was based on negative and unsatisfactory evidence, *viz.*, his failure to isolate glucosephenylosazone from phenylhydrazine and potassium glucosate. Marchlewski did not describe his experiments in detail, but it may be pointed out that pure phenylhydrazine and pure glucose do not yield the osazone under normal conditions, acidification with acetic acid being necessary.

Hönig and Rosenfeld (*Ber.*, 1877, **10**, 871) claimed to have isolated the compound $C_6H_{11}O_6Na$ from glucose and sodium ethoxide in alcoholic solution. Zemplén and Kunz (*Ber.*, 1923, **56**, 1705) reopened the question and pointed out that attempts to use the compound in synthetic experiments, notably by Skraup and Kremann (*Monatsh.*, 1901, **22**, 1040), had failed. The existence of an alcoholate structure was therefore considered to be doubtful, and Zemplén showed that an addition compound between glucose and sodium ethoxide, $C_6H_{12}O_6,NaOC_2H_5$, appeared to be formed, the presence of the ethoxide residue being established by a qualitative test.

This work has now been repeated and *compounds* of the type $C_6H_{12}O_6,NaOR$ have been isolated from glucose in absolute-alcoholic solution. If a trace of water was present, however, the compounds isolated contained no combined alcohol, which may explain the results of the earlier workers. From potassium hydroxide and glucose, the *compound* $C_6H_{12}O_6,KOH$ may readily be isolated, which is probably similarly constituted to the sodium alkoxide compounds. By analogy, therefore, it appears that potassium glucosate is an addition compound of potassium hydroxide and glucose, and this is supported by the analytical results. No evidence can be secured for the existence of any other compound in the range of concentrations studied. It has been found possible, however, to indicate the probable position of the addendum. By a single treatment of potassium glucosate with pure methyl sulphate under mild conditions, apart from unchanged glucose, crystalline tetra-acetyl β-methylglucoside may be obtained, together with a syrup which is evidently a mixture of this with the α-isomeride. All the methoxyl is glucosidic, there being no evidence of any further substitution of the glucose molecule by methyl groups.

Hence, one is entitled to assume that the reducing group is involved in the combination between sugar and alkali, as might have been expected from general considerations. If the alcoholate formula, $C_6H_{11}O_6K$, were the true one, a more complete conversion into the methylglucosides would have been expected, but the isolation of so much unchanged glucose lends support to the theory that the attachment between glucose and alkali is relatively weak, and is probably to be ascribed to co-ordination.

The fact that α- and β-methylglucosides form no addition compounds with potassium hydroxide, or that the glucosides examined by Mackenzie and Quin (*loc. cit.*) form no derivatives with the alkaline-earth hydroxides, renders it difficult to imagine that the oxygen atom of the reducing group can act as a donor of electrons to the metal atom concerned. If, however, we suppose that the hydrogen atom of that group acts as an acceptor of electrons from the oxygen atom of the alkali hydroxide or alkoxide, the known facts are explained (see inset). This is in agreement with the well-known fact that alkaline hydroxides appear to form very stable monohydrates, which is attributed



to hydration of the negatively charged hydroxyl ion HOH ⟵ OH', the ion acting as a powerful donor of electrons (Sidgwick, "Electronic Theory of Valency," Oxford, 1927). The suggestion is therefore made that the compounds under review are of this general type.

The reducing disaccharide cellobiose (glucose-β-glucoside) forms an addition compound $C_{12}H_{22}O_{11}$,KOH in which it is clear that the reducing group is involved, since crystalline β-methylcellobioside hepta-acetate may be isolated by suitable treatment with methyl sulphate. The situation is complicated by the fact that cellobiose appears to form in addition $C_{12}H_{22}O_{11}$,2KOH, which implies that at least two positions in the molecule are available for the attachment of potassium hydroxide residues. This has been confirmed by the isolation of a monomethyl methylcellobioside, the structure of which is being investigated to determine the position of the second methyl group.

Even more complex is the case of maltose, which would appear to form a tri- as well as mono- and di-potassium hydroxide derivatives; and a dimethyl methylmaltoside has been isolated confirming this view.

It is not thought probable that during the treatment with methyl sulphate the alkali residue would migrate to a new position in the sugar group, thus causing the introduction of the methyl residue in a position different from that originally concerned. The possibility has, however, been kept in mind, and minimised as far as possible by the avoidance of the presence of water during methylation. It may be pointed out, however, that even if water or other ionising solvents were present, the method would still be expected to indicate those hydroxyl groups of maximal acidity in the sugar molecules examined, because, whether they are closely bound to alkali or not, it is not likely that the centres of acidity will change during the operation.

In the case of disaccharides, therefore, hydroxyl groups other than the reducing group appear to unite with potassium hydroxide. This is to be expected because of the formation of the important and apparently well-marked series of saccharates from sucrose and alkali and alkaline-earth hydroxides. The structure of these more complex addition compounds is being studied in detail.

Experimental.

Typical Preparations of Potassium Hydroxide–Glucose.—(1) *From penta-acetyl glucose.* β-Penta-acetyl glucose (5 g.) was moistened with absolute alcohol (10 c.c.), and a solution of potassium hydroxide (6 g.) in alcohol (60 c.c.) added; after an hour the insoluble product was filtered off, washed first with alcohol and finally with ether, and dried in a vacuum over phosphoric oxide [Found: KOH, by titration with $N/10$-H_2SO_4 to phenolphthalein, 23·6; $C_6H_{12}O_6$, by treatment with alkaline hypoiodite (Bergmann and Machemer, *Ber.*, 1930, **63**, 316), 75·1. $C_6H_{12}O_6$,KOH requires KOH, 23·7; $C_6H_{12}O_6$, 76·3%].

(2) *From glucose.* *d*-Glucose (10 g.) dissolved in water (10 c.c.) was mixed with alcohol (150 c.c.), and alcoholic potassium hydroxide (50 c.c., 8%) added, and the precipitated derivative was treated as in (1) (Found, by foregoing methods: KOH, 24·0; glucose, 75·2%).

Sodium Ethoxide–Glucose.—Ethyl alcohol was dried by distillation over quick-lime and twice over sodium. Glucose (1 g.), dried over phosphoric oxide, was dissolved in dry alcohol (100 c.c.), and a solution of sodium ethoxide in alcohol (0·4 g. Na in 10 c.c.) added. The precipitate was rapidly collected in a funnel protected from moisture and carbon dioxide by means of a soda–lime tube, washed with alcohol, and dried, at first at room temperature over phosphoric oxide in a vacuum and then for 24 hours at 60° under the same conditions [Found: NaOEt (titration), 27·8; OEt (Zeisel), 16·0; $C_6H_{12}O_6$ (Bertrand), 66·9. $C_6H_{12}O_6$,$NaOC_2H_5$ requires NaOEt, 27·4; OEt, 18·1; $C_6H_{12}O_6$, 72·6%]. A similar preparation made from penta-acetyl glucose had similar properties.

Sodium Methoxide–Glucose.—This was prepared from penta-acetyl glucose (5 g.) in admixture with sodium methoxide in methyl alcohol (2 g. Na in 25 c.c.). The precipitate was washed with methyl alcohol, in which it was slightly soluble, and finally with dry ether, and dried as for the ethoxide [Found: NaOMe (titration), 22·7; $C_6H_{12}O_6$ (hypoiodite), 77·4; OMe (Zeisel), 13·0. $C_6H_{12}O_6$,$NaOCH_3$ requires NaOMe, 23·1; OMe, 13·2; $C_6H_{12}O_6$, 76·9%].

The Formation of Potassium Hydroxide–Glucose under Different Conditions.—A rough estimate of the amount of potassium hydroxide taken up by the glucose molecule can be obtained

by treating a known volume of alcoholic potassium hydroxide of known strength with a known volume of a glucose solution in 80% alcohol. By filtration through a Gooch crucible a solution can be obtained, titration of which indicates how much alkali has been withdrawn by the sugar. It is necessary to assume that no significant volume changes have taken place and also that the addition compound is not appreciably soluble in this equilibrium solution. The solid in the crucible is drained and washed once with alcohol to remove adhering liquid; solution in water and titration with standard acid then gives a direct estimate (method 2) of the combined alkali, which, however, is probably less accurate than the value obtained by the indirect method (method 1). Typical results are as follows :

Total concn. of glucose, %		1·01	1·78	2·51	4·32	4·81
Concn. of KOH, *N*	Initial	0·75	0·51	0·30	0·29	0·27
	Final	0·69	0·40	0·16	0·04	0·03
KOH combined, %	Method (1)	33·6	31·8	32·1	32·6	28·7
	,, (2)	31·9	32·5	32·4	30·7	29·6

Since 100 g. of glucose require 31·1 g. of potassium hydroxide to form a 1 : 1 compound, it would appear that such a compound is formed within the range studied.

Formation of More Complex Compounds from Cellobiose and Maltose.—The results with these sugars are tabulated below. For a compound of the type $C_{12}H_{22}O_{11}$,KOH, 100 g. of the disaccharide require 16·4 g. of potassium hydroxide. Evidently in these cases this simple compound may be formed only in dilute solution, but it is possible that small quantities of the more complex derivatives are produced at the same time. In the case of cellobiose the composition approximates to $C_{12}H_{22}O_{11}$,2KOH, and for maltose in the more concentrated alkaline solutions $C_{12}H_{22}O_{11}$,3KOH appears to exist. The variations between the results of the two methods are due to the decomposition, by washing with alcohol, of the higher addition compounds, and illustrate the instability of these more complex derivatives.

		Cellobiose.				Maltose.			
Total concn. of sugar, %		0·88	1·49	1·9	1·8	1·6	0·9	1·5	2·1
Concn. of KOH, *N*	Initial	0·76	0·65	0·15	0·075	0·4	1·0	0·92	0·15
	Final	0·69	0·58	0·080	0·02	0·31	0·92	0·80	0·043
KOH combined, %	Method (1)	32·9	26·0	21·4	17·1	36·2	44·5	45·6	28·5
	,, (2)	23·0	19·5	21·4	16·0	36·0	36·8	37·0	25·1

Potassium Hydroxide–Glucose and Methyl Sulphate.—The dry compound (13 g.) was stirred with dry, neutral methyl sulphate (120 c.c.) for 5 minutes at 45° and for 5 minutes at 70°, the liquid was then removed, the product washed with acetone, and dissolved in hot methyl alcohol (100 c.c.). On cooling, crystals of potassium methyl sulphate separated which were filtered off, and a solution of potassium hydroxide (3 g.) in alcohol (20 c.c.) was added, followed by ether (700 c.c.). By this means most of the potassium glucosate so produced was recovered (9·5 g.), whilst there remained in solution that portion of the starting material which had undergone reaction with the methyl sulphate.

Isolation of tetra-acetyl β-methylglucoside. After acidification with acetic acid and removal of the solvent, the syrup was acetylated by treatment for 2 hours with acetic anhydride (30 c.c.) and anhydrous sodium acetate (5 g.). The mixture was poured into water, the solution neutralised with sodium bicarbonate, and extracted with chloroform. Removal of the solvent (diminished pressure) after drying with sodium sulphate yielded a syrup which, on treatment with alcohol, partly crystallised in the prisms characteristic of tetra-acetyl β-methylglucoside (0·9 g.); this was non-reducing, had m. p. 102° (not depressed on admixture with an authentic specimen prepared from acetobromoglucose), and $[\alpha]_D^{20°} = -20°$ in chloroform (*c*, 2·5) (Found : OMe, 8·5; $CH_3{\cdot}CO$, 47·0. Calc. for $C_{15}H_{22}O_{10}$: OMe, 8·6; $CH_3{\cdot}CO$, 47·5%).

The non-reducing syrupy residue from which this crystalline derivative had been extracted weighed 3·0 g.; $[\alpha]_D^{20°} = +20°$ in chloroform (*c*, 1) (Found : OMe, 8·6; $CH_3{\cdot}CO$, 46·9%). It was assumed therefore to consist of a mixture of α- and β-methylglucoside tetra-acetates, but a further investigation was carried out to see if any substitution whatever had occurred in other parts of the glucose molecule.

Examination of the syrupy glucoside. Deacetylation was carried out with sodium methoxide (Zemplén, *loc. cit.*), and the resulting aqueous solution was hydrolysed by dilute sulphuric acid (6%) at 90° for 7 hours. After neutralisation by means of barium carbonate, the solution was evaporated under diminished pressure, yielding a syrup which was insoluble in absolute alcohol (cf. glucose). To the solution in 80% alcohol, excess of alcoholic potassium hydroxide was

added, and the thick precipitate of potassium hydroxide–glucose was collected and dried (1·5 g. Calc., 1·9 g.). The residual solution was acidified with acetic acid and treated with phenylhydrazine (0·5 g.) at 90° for 30 minutes. On cooling, phenylglucosazone separated (0·1 g.); recrystallised from aqueous alcohol it had m. p. 204°, and contained no methoxyl residue (Zeisel). It followed therefore that the original syrup was only substituted by methoxyl in the reducing group, the formation of the osazone being due to the slight solubility of potassium hydroxide–glucose. No evidence could be obtained of the formation of addition compounds between potassium hydroxide and α- and β-methylglucosides.

Potassium Hydroxide–Cellobiose.—Cellobiose octa-acetate, prepared by the method of Haworth and Hirst (J., 1921, **119**, 193), was deacetylated after Zemplén (*loc. cit.*) to yield the free sugar, of which 2 g. were dissolved in 80% ethyl alcohol (50 c.c.), alcoholic potassium hydroxide (100 c.c., 0·08*N*) being added. The product was washed rapidly with alcohol (20 c.c.) and dried in the usual way [Found: KOH (titration), 13·8; $C_{12}H_{22}O_{11}$ (iodine), 86·0. Calc. for $C_{12}H_{22}O_{11}$,KOH: KOH, 14·1; $C_{12}H_{22}O_{11}$, 85·9%]. The white powder was similar in properties to the glucose derivative.

Reaction with Methyl Sulphate.—The dry product (2 g.) was stirred with dry, neutral methyl sulphate for 8 minutes at 60°. Coagulation ensued, the reagent was removed, the product washed with acetone, and dissolved in hot methyl alcohol (40 c.c.). On cooling, the precipitated potassium methyl sulphate was removed, and unchanged cellobiose was precipitated by the addition of potassium hydroxide (0·5 g.) in alcohol (10 c.c.). The recovered product weighed 0·9 g. After acidification with acetic acid, the solvent was removed (diminished pressure) and the syrup subjected to acetylation by acetic anhydride (10 c.c.) and sodium acetate (1 g.) at 100° for 30 minutes.

Isolation of Hepta-acetyl β-Methylcellobioside.—The acetylation mixture was poured into water, and the solution neutralised with sodium bicarbonate before extraction with chloroform. Removal of the solvent after drying over sodium sulphate yielded a faintly reducing syrup (1·4 g.) which crystallised on treatment with alcohol giving hepta-acetyl β-methylcellobioside (0·7 g.); m. p. 178°, $[\alpha]_D^{15°} = -23·0°$ in chloroform (*c*, 1·6) (Found: C, 50·0; H, 6·0; OMe, 4·7. Calc. for $C_{27}H_{38}O_{18}$: C, 49·9; H, 5·9; OMe, 4·9%). The residual syrup showed $[\alpha]_D^{15°} = -1°$ in chloroform (*c*, 1·1) (Found: OMe, 5·4; CH_3·CO, 45·9. Calc. for $C_{27}H_{38}O_{18}$: CH_3·CO, 46·3%). It would appear therefore to consist of a mixture of α- and β-glycosides.

The use of dilute alcoholic potassium hydroxide appears, then, to favour the production of a compound of the type $C_{12}H_{22}O_{11}$,KOH in which the reducing group and the hydroxide are intimately connected.

When, however, preparations using more concentrated alkali (see table, p. 1163) are treated with methyl sulphate, more complex methylated derivatives may be isolated, in particular a monomethyl methylcellobioside, the structure of which is being investigated. Even more interesting is the case of maltose which can be made to afford methylmaltosides, monomethyl methylmaltosides, and dimethyl methylmaltosides, as might be expected from a consideration of the table on p. 1163.

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