PROGRESS IN THE STRUCTURAL STUDY OF CARBOHYDRATES[1]

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My first words must be of the debt I owe to my fellow-chemists in America who have paid me the singular compliment of adding my name to the list of Willard Gibbs Medallists. It is the custom, I know, to mark the presentation of the medal by the delivery of an address on the researches for which the award is made, and custom cannot be defied; but if for a moment I may be permitted to speak of my feelings rather than of my work, it would be to say that this honour has never been bestowed on one who prized it more than I do. To me, the distinction carries a double significance, for I regard it as an outward symbol of the personal friendship extended to me by the chemists of America, a friendship which I count as not the least of fortune's favours, and I esteem this award in even higher measure through its association with the name of Willard Gibbs.

It would be ungracious of me, also, if I failed to take this opportunity of expressing the gratitude I have always felt to the scientists of the New World for another gift, one which came long ago, yet gradually and almost imperceptibly. I refer to the encouragement, denied alas to so many who are worthy of it, which comes to a scientific worker when he finds, as I found in this country, that his work is closely studied, and that, having been weighed in the balance, it is not found wanting. I can never forget the stimulus, the exaltation, derived from your understanding and sympathetic interest in the problems with which I am engaged. The lot of the investigator is often made lonely through lack of a word of encouragement, but, throughout

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[1] An address delivered on the occasion of the Willard Gibbs Medal Award, Chicago, Illinois, September 17, 1926.

life, it has been my good fortune to be surrounded by indulgent friends.

I turn now to my address, finding comfort in the fact that organic chemists are deemed worthy of the Willard Gibbs Medal, and that some of my predecessors share with me the misfortune that their activities have lain in fields remote from that vast country opened out by the instinctive genius of the man whose memory we honour to-night. It is, indeed, a far cry from the Structure of Carbohydrates to the Phase Rule, yet although there may be no very obvious connexion between them, a relationship is nevertheless there, for the work I hope to summarise for you could not have been contemplated, far less accomplished, without daily application of the principles laid down by the physical philosopher of New England. The year in which the classical papers of Willard Gibbs appeared was the year of my birth; his views had reached the lecture room before I vacated the students' bench and, throughout my reseach life, I have been under the influence he radiated on British science. It is a debt of which I am fully conscious, and I add my tribute to his inspiration.

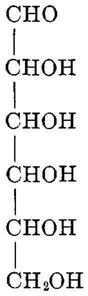
This is the 17th of September, 1926, and memory warns me that, exactly a quarter of a century ago to a day, I commenced the first of what has proved to be a continuous series of investigations on the chemistry of sugars. In the intervening years I have often been asked why my attention was drawn to this particular class of compound, and this is a question which might well be put to many others, for it must be evident, even to the casual student of organic chemistry, that there has been in recent years a marked expansion in the study of carbohydrates. Whereas at the beginning of the century there were perhaps a dozen expert workers in this field, their successors are now legion, and the number grows almost daily. It has been an amazing development, and one for which it is difficult to find an adequate explanation, but, looking back on my own experience, I think I can discern the underlying reasons. Certainly among these reasons, simplicity can find no place, either in respect of manipulation or of speculation. Even in the investigation of the compounds which, with unconscious irony, we term the "simple" sugars, the organic chemist is confronted with formidable experimental difficulties, to be appreciated fully only by those who have submitted themselves to this stern discipline. Months may be spent in attempts to induce a syrup to crystallise; and, even if this be successfully accomplished, the product may prove to be one of twenty distinct but closely-related isomeric forms. Nor can the prospect of material reward play any considerable part in attracting workers to the chemistry of sugars, for, so far at least, our sugar factories and even our cellulose industries depend only in minor degree on the power to elucidate molecular constitution. We must therefore look for other causes, specific to the chemistry of sugars, which invest these compounds with a special fascination. My reflections have convinced me that first importance must be attached to the fact that carbohydrates are essentially the products of natural, as opposed to artificial, synthesis. Within us and around us, through the agency of life, these compounds are being built up and broken down, formed and transformed, finally to be consumed in the fire of metabolism. To study the sugars is, in brief, to study the great molecular channel through which solar energy flows to us. No wonder the thought of such work makes a powerful appeal to the student of vitalism who is naturally somewhat inclined to turn aside from that aspect of chemistry which deals with purely artificial reactions. Then again, one must recognise that to be an intelligent investigator of the sugars the chemist must be something of a biologist and of a physiologist, but, above all, he must be a physicist. This diversity of interest finds its expression, not only in the library but also in the laboratory where one day our chemist may be manipulating many kilograms of plant products only to turn later to the other end of the working scale to examine a few milligrams of a precious crystalline sugar. He has to be familiar with bacteriological, as well as with orthodox chemical processes; he finds himself engulfed in the mysteries of colloids and, at all times, the precision instruments of the physicist are his daily tools. Taking a broad and, I trust, an impartial view of these requirements, the marvel to me is not so much that many investigators turn to the sugar group, but that so many can resist its attractions.

There remains another factor, as potent, I believe, as any I have mentioned, and that is expressed in the statement that to-day the sugars are being formulated in terms of molecular structure, so that at last we are approaching the position when we may study their reactions with precision and understanding.

There is a special satisfaction in discerning how our knowledge of carbohydrates has expanded in response to the stimulus conveyed by fresh views on structure. So long as glucose was regarded merely as a molecule $C_6H_{12}O_6$, characterised mainly through its power to reduce Fehling's solution and to rotate the plane of polarised light to the right, the rôle of the compound in natural processes, of necessity, remained obscure. An entirely new situation was created when we began to recognise that within the glucose molecule there are five hydroxyl groups, and a further step was taken when it became apparent that these groups varied in character and property.

GLUCOSE

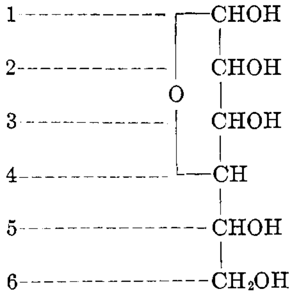
This brings me to a point where I may indicate the general position which had been reached in 1901 when, as I have indicated, my share in this work commenced. The first phase of Fischer's classical researches was over; the most important monosaccharides had been synthesised, and the principles of stereoisomerism had been made the basis of a rational classification which accounted for all the hexoses and pentoses then known. Nevertheless the chemistry of sugars was then in a state of flux. Limiting our attention meanwhile to the fundamental case of glucose, a sugar which I believe to be the origin of all carbohydrates, the old aldehydic formula for the compound had been replaced by a cyclic structure in which an oxygen ring was represented as connecting the first and fourth carbon atoms as shown below:



$CHO-CHOH-CHOH-CHOH-CHOH-CH_2OH$

Aldehydic or Straight Chain formula for glucose

I



Butylene-oxide formula for glucose

II

I need not enumerate here the reasons which led to the opinion that the reducing sugars should be formulated as cyclic compounds, but may perhaps state that the adoption of the five-membered ring was dictated largely by analogy, rather than by direct experimental proof. Nevertheless, the adoption of such a formula marked a substantial advance. For example, it was thereafter possible to recognise that the hydroxyl groups of a reducing sugar such as glucose may be classified into three categories: (*a*) The terminal hydroxyl group attached to carbon atom No. 1, which, being readily oxidised, is responsible for the reducing action on Fehling's solution. This group is feebly acidic in character, and is not only highly reactive, but is also capable of transposition giving rise to the α- and β-forms of the sugar. (*b*) The hydroxyl groups within the ring. These are characterised by less abnormal properties and remain rigidly fixed in position, thereby accounting for the stereochemical distinction between the isomeric aldoses. (*c*) The hydroxyl groups external to the ring, the most important being the primary alcohol group, which takes part in the oxidation reactions leading to the formation of such compounds as the glycuronic acids.

I have given only an indication of the advantages secured through classification of the hydroxyl groups in glucose, but possibly I have said sufficient to indicate that the complete exploration of a sugar molecule demands the study of the individual asymmetric systems, for it must be remembered that each contributes its own specific properties to those of the molecule as a whole.

Evidently twenty-five years ago the time was opportune to engage in this highly specialised study of sugar structure, but even more attractive problems were presented by the prospect of opening out a path whereby the molecular constitution of disaccharides and polysaccharides could be approached. I need not remind you that the disaccharides may be regarded as compound sugars formed by the union of two hexoses through loss of a molecule of water. When it is considered that some disaccharides are devoid of action upon Fehling's solution, it is at once clear that the reducing groups of each of the constituent molecules must have taken part in the formation of the complex sugar. Similarly, in the case of reducing disaccharides, it is evident that the reducing groups of only one of the constituent glucose molecules is involved in the union, whilst the other remains free. This much was known, but, beyond that, all ideas of the exact positions through which one sugar united with another to form a disaccharide were purely speculative. The great master Emil Fischer clearly recognised this limitation imposed by the lack of suitable experimental processes, and abstained from anything more than tentative suggestions as to the molecular structure of the disaccharides. Would that his caution had descended on his successors.

With regard to polysaccharides, the situation at the beginning of the century was even more obscure. Within the single empirical formula $(C_6H_{10}O_5)_n$ were accommodated such diverse compounds as cellulose, starch and glycogen, but beyond the knowledge that, as a purely arithmetical ratio, three hydroxyl groups were present for each six carbon atoms, remarkably little evidence was available which could be brought to bear on the problem of molecular constitution. To sum up, the position was that structural sugar chemistry ended with the monosaccharides.

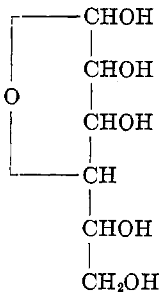
METHYLATED SUGARS

I have outlined some of the carbohydrate problems which then confronted the chemist, and it is my belief that the most effective method of solving these structural mysteries has been found in the study of methylated sugars. It was with this hope in my mind that I commenced my work. A word of explanation is perhaps necessary as to what is meant by the expression "methylated sugar." Clearly, if the sugars are poly-alcohols, they should be capable of forming the corresponding ethers, and these should display the stability, particularly towards hydrolysts, which is so characteristic of the ether type. A first step in this direction had been taken by Emil Fischer who discovered the general reaction of converting reducing sugars into the corresponding methylglucosides. In such compounds, however, the methyl group is present in the terminal position, and is readily lost on hydrolysis. The alkyl glucosides are therefore more closely akin to esters than to ethers. It was evident that, if normal poly-ethers of the sugars could be obtained, the key would be found to decipher the complete structure of the complex sugars. Let us take an example. In maltose, we have two glucose residues attached by the reducing group of one of them to a non-reducing group of the other. There are, however, four such groups available and the question is, which of them is involved in the coupling? It is the case that the hydroxyl positions in the disaccharide may be substituted by acetyl or other acyl groups, and that the point of junction between the constituent hexose residues cannot take part in such substitution. But, if we disrupt the acetylated disaccharide by means of hydrolysis, we eliminate at the same time the substituting acyl groups, and thus all structural evidence is lost. An entirely different state of affairs was to be expected in the case of a methylated disaccharide, as the methyl groups would survive hydrolysis and hydroxyl groups would be regenerated in the positions through which the constituent sugars had been joined together. Thus, in the case of maltose, for example, we should obtain as the hydrolytic products a tetramethyl glucose and a trimethyl glucose. In one of these methylated sugars only a reducing hydroxyl group can be present, (Let us call it A), whilst in the other there must be, in addition, one non-reducing hydroxyl group (Let us call it B). Obviously, we would then be in the position of regarding maltose as a compound sugar consisting of two glucose residues condensed together through the positions we have indexed as A and B. The principles I have enunciated are capable of general application, and can be extended to the constitution of glucosides and polysaccharides, but the exploitation of the idea demanded that much preliminary research had to be undertaken. The first consideration was to find a method of alkylation suitable to the sugar group, and the other was the preparation of a sufficiently large variety of fully and partly methylated sugars which would function as reference compounds in extending the general research scheme. Fortunately, a convenient experimental process of methylation lay in my hands. You will remember the elegant method discovered by Purdie, my distinguished predecessor in the Chair of Chemistry at St. Andrews, by means of which remarkably smooth alkylation is effected by submitting hydroxy-compounds to the joint action of methyl iodide and dry silver oxide. In applying this process to the particular case of reducing sugars complications were introduced through the oxidising effect of the silver oxide, but these were overcome by the use of methylglucoside as the starting material. This compound was successfully converted into a tetramethyl methylglucoside which, following the general rule, lost only the glucosidic group on hydrolysis and, accordingly, yielded a tetramethyl glucose as the final product. This was the first methylated sugar obtained and, as originally formulated, the compound was represented as a butylene-oxide. In the meantime, this formula may be retained, although the general question of the different positions assumed by the oxygen-ring in glucose must engage our attention at a later stage. Tetramethyl glucose represents only one type of a methylated sugar, but other types containing a smaller number of alkyloxy-groups are known. Of these, we may select as an example, one particular variety of monomethyl glucose. Commencing with glucose-diacetone, a compound in which four hydroxyl groups of the sugar are already substituted, methylation can give, as a maximum, only a monomethyl derivative. Subsequent hydrolysis removes the unstable acetone residues, but leaves the methyl group unaffected, and a monomethyl glucose results. This may be taken as representative of the general method employed to prepare other partly methylated sugars of which numerous examples are now known. Some of the properties of methylated sugars may be mentioned at this stage as, in many respects, they differ profoundly from those of the parent compounds. As is to be expected, the step-by-step replacement of hydroxyl by methoxyl results in a progressive increase in stability and solubility, so that in the case of tetramethyl glucose, the sugar dissolves in all ordinary solvents, ranging from water to petroleum ether. The compound is also so stable that it can be distilled quantitatively in a vacuum.

Characterised as they are by such convenient properties, methylated sugars have proved of great value in the detailed study of such problems as mutarotation and the determination of the molecular rotations of the α- and β-forms of sugars. Further, their use enables the investigation of sugars to be confined to selected hydroxyl groups, thereby revealing the characteristic properties of each asymmetric system in the molecule. The fascination of methylated sugars is such that one might have continued to explore their properties to the exclusion of the ultimate object in view in preparing such compounds.

THE OXYGEN RING

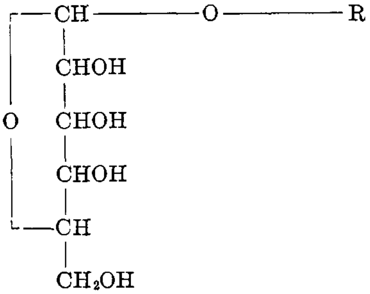
This brings me to the results obtained in the application of methylated sugars to the structural problems of complex carbohydrates, but, as a preliminary to this discussion, let us glance for a moment at the question of the position occupied by the oxygen ring in the simple sugars. As I have already explained, the view originally held was that glucose is definitely a butylene-oxide.



That the ring does not, of necessity, remain in one fixed position was revealed about twelve years ago, when Fischer isolated a form of methylglucoside which differed from the known α- and β-varieties. Following the general but regrettable custom, the new product was termed by him "γ-methylglucoside," and he recorded that, when hydrolysed, it yielded ordinary glucose. Application of the methylation process at once gave the explanation as to why methylglucoside should exist in a larger number of forms than stereochemical considerations accommodate, as a new variety of tetramethylglucose utterly distinct from the crystalline sugar to which I have referred was thereby produced. Consideration will show that the only possible reason for the existence of these isomeric tetramethyl glucoses must lie in the different positions assumed by the oxygen ring in each compound. The introduction of the methyl groups prevents the ring from reverting to a stable from an unstable position, and thus methylated sugars are invested with increased importance, and acquire a new sphere of usefulness.

The expression "γ-sugar" crept into use to designate aldoses and ketoses in which the internal ring is displaced from the normal stable position, and has therefore been applied to an entirely new, highly reactive class of sugars. In view of these results, I would urge for extreme caution in accepting any suggestion that glucose is definitely an amylene-oxide. There can be no fixed and unalterable structure for glucose, as it is conceivable that the oxygen-ring may couple position 1 with any of the five remaining positions, so that if we include an aldehydic variety, d-glucose may actually exist in eleven forms and may react as a mixture of any of these. It is not a case of one oxygen-ring, but the option of several alternatives and, in consequence, there can be no formula universally applicable to glucose. It follows also that the ring-structure of a sugar must be investigated separately in each derivative studied.

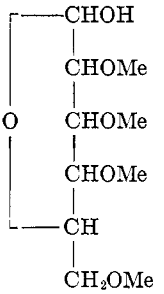
Examples are now available in which this has been done. Recently, it has been shown that, when tetramethyl glucose is oxidised, a trimethoxy-glutaric acid is found amongst the oxidation products. If this be accepted, the methylated sugar must be an amylene-oxide and the constitution of certain natural glucosides, such as salicin, arbutin and indican, must conform to the following structural type:



III

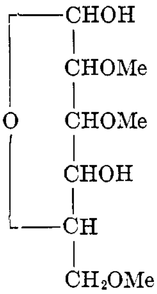
Beyond this it is inadvisable to go.

Although a considerable variety of methylated hexoses were prepared as a preliminary to the constitutional study of di- and polysaccharides, it has been ascertained in practice that only five of these find application. The sugars in question are: tetramethyl glucose, two isomeric forms of trimethyl glucose, tetramethyl γ-fructose and tetramethyl galactose. The compounds are formulated below, but it is perhaps necessary to emphasise that only in the cases of tetramethyl glucose and of tetramethyl galactose are we justified in allocating a fixed position to the internal oxygen ring.



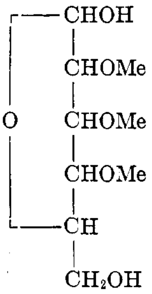
2:3:4:6-Tetramethyl glucose or galactose.

IV



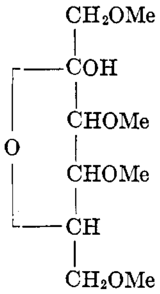
2:3:6-Trimethyl glucose.

V



2:3:4-Trimethyl glucose.

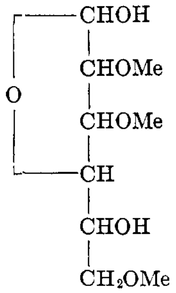
VI



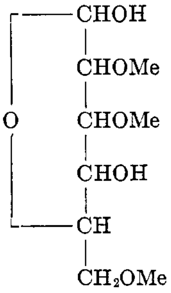
Tetramethyl γ-fructose.

VII

Greater complications are encountered when we consider the two trimethyl glucoses. In one of these sugars (2:3:4-trimethyl glucose), which is a colourless liquid resembling glycerol in appearance, the unsubstituted hydroxyl group is definitely in the No. 6 position, and the formation of this compound from a disaccharide is proof that the one hexose residue is attached to the terminal position of the other. The case presented by the isomeric 2:3:6-trimethyl glucose is more complex, as the oxygen ring undoubtedly fluctuates between the positions 1:4 and 1:5, thereby giving us the two alternative compounds represented by the following formulae:



VIII



IX

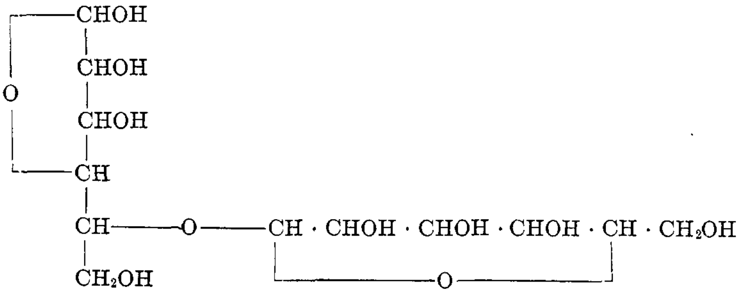
Notice that this disconcerting property at once introduces an ambiguity into structural studies, in that the formation of this sugar from a disaccharide is not discriminative, as the coupling of the constituents may be through either position 4 or position 5. It is necessary to make this point clear as, in the structural classification of disaccharides built up by Haworth, this important issue has been entirely overlooked. In addition, it is now recognised that his work includes a fundamental experimental error, which renders his whole scheme valueless, and that, consequently, so far as the constitution of disaccharides is concerned we are only at the beginning of things in place of at the end.

THE DISACCHARIDES

Let us turn to the authentic results obtained by applying the methylation process to disaccharides. The first point to notice is that two of the diglucoses are found to behave in exactly the same way as shown below:

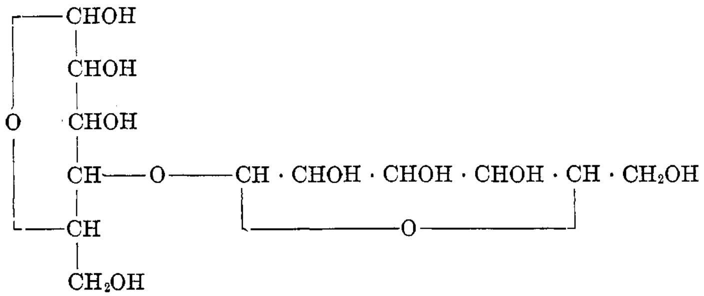
$$\begin{array}{c} \text{Maltose} \\ \text{and} \\ \text{Cellobiose} \end{array} \xrightarrow[\text{and hydrolysis}]{\text{on methylation}} \begin{array}{c} \text{2:3:4:6-Tetramethyl glucose.} \\ \text{and} \\ \text{2:3:6-Trimethyl glucose.} \end{array}$$

On first inspection this result might be interpreted as meaning that in maltose and in cellobiose the glucose residues are attached through the same positions, the one sugar being α-glucose-glucoside, and the other β-glucose-glucoside. Such is not the case, however, and it has to be remembered that we must also accommodate *iso*-maltose and *iso*-cellobiose within our structural scheme. It is impossible to do so unless we take into account the fact that 2:3:6-trimethyl glucose could arise from either of the following diglucoses:



Glucose 5-glucoside.

X

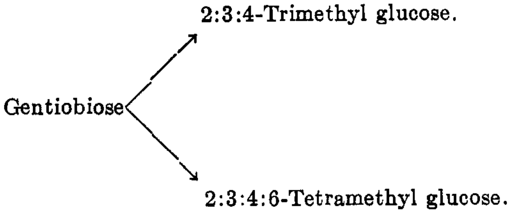


Glucose 4-glucoside.
XI

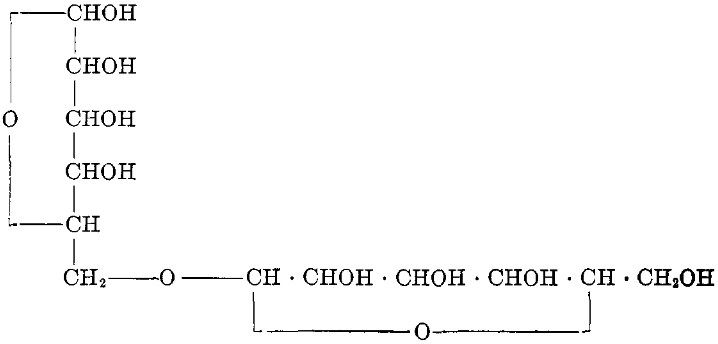
We are thus forced to the conclusion that one of these formulae represents the pair maltose and *iso*-maltose, whilst the other is reserved for cellobiose and *iso*-cellobiose. The same ambiguity is attached to the case of lactose, as the compound yields 2:3:4:6-tetramethyl galactose together with 2:3:6-trimethyl glucose. In consequence, if we regard the non-reducing section of the formulae given above as representing galactose, this residue may be attached either to position 4 or 5 of the glucose component. The situation, apparently uncertain, is greatly simplified when we remember that good reasons exist for the belief that starch and glycogen are composed of γ-glucose residues. This demands that maltose must be represented by Formula X in which case we are in a position to allocate Formula XI to cellobiose.

To the mass of evidence already available in support of this view, may be added the results recently contributed by Zemplén who, having degraded cellobiose to a gluco-erythrose, found that the product failed to form an osazone. This observation is consistent with the allocation of a glucose 4-glucoside structure to cellobiose as shown in Formula XI.

A clearer issue is discernible when we consider the case of gentiobiose, as this sugar, on methylation and hydrolysis, behaves as synopsised below:



In this case, there is no reason to doubt the fact that the hydrolytic products have been correctly identified and, further, the result is free from the complication of a dual interpretation, so that the structure of the parent disaccharide becomes:

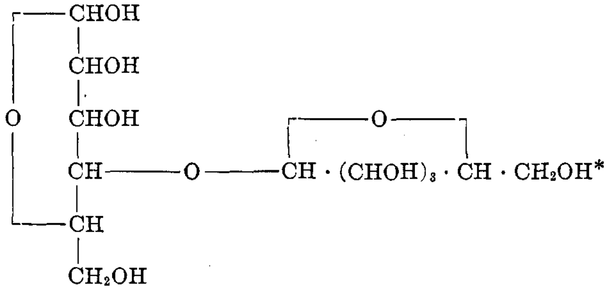


XII

It is particularly gratifying to add here that one of the most recent results obtained by the American school of carbohydrate chemists has proved that the synthetic diglucose prepared by Fischer and termed by him "iso-maltose" is identical with gentiobiose, and thus possesses the above structure. This observation is important, as it will have the effect of removing another confusion from sugar chemistry through the deletion of the name "iso-maltose" for a disaccharide which is in no way related to the *iso*-maltose obtainable from starch.

Taking into account much supplementary evidence, a review of the whole situation shows that the reducing disaccharides based on glucose can be relegated to three structural types:

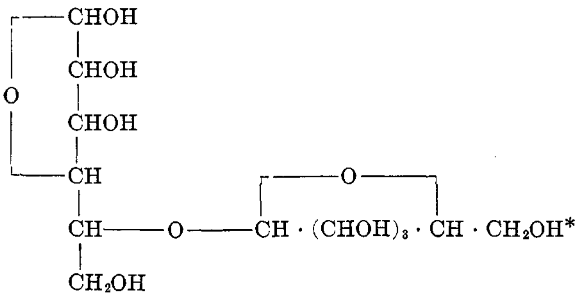
A



XIII

A represents cellobiose and *iso*-cellobiose, and where * = galactose, also formulates lactose

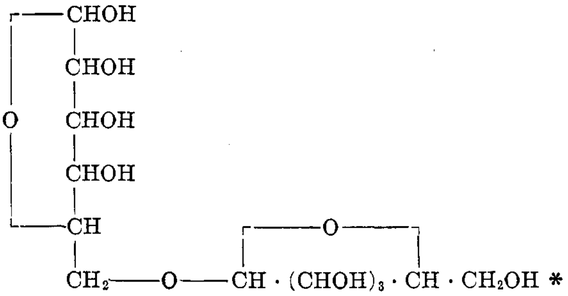
B



XIV

B represents maltose and *iso*-maltose, and where
* = galactose also formulates melibiose

C



XV

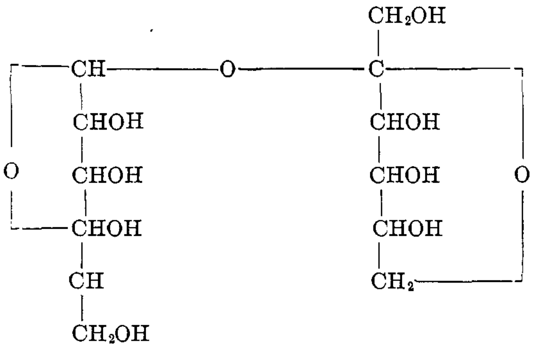
C represents gentiobiose

So far I have left untouched the important case of sucrose, partly on account of the difficulty involved in clearing up a problem which, in itself sufficiently complicated, has been rendered more confused through the acceptance of results based on faulty work. It is probably the best known fact in sugar chemistry that sucrose may be hydrolysed to give dextro-rotatory glucose and laevorotatory fructose, but this has led to the idea that these constituents exist in the disaccharide in precisely the same form in which they are finally isolated. It is nevertheless the case that the fructose component of sucrose is not the ordinary laevorotatory variety of the sugar, but the unstable form into which the sugars may pass through transposition of the oxygen ring. In other words, this component is γ-fructose.

This was the first serious theoretical complication encountered by Purdie and myself twenty-three years ago for, naturally, sucrose was the first disaccharide to which we turned our attention in developing the research scheme. An octamethyl sucrose was duly obtained and hydrolysed; the ordinary crystalline tetramethyl glucose was readily forthcoming as one of the scission products, but the fraction containing the tetramethyl fructose proved to be a liquid and, contrary to our expectation, was *dextro-rotatory*. This result can now be expressed in terms of the scheme

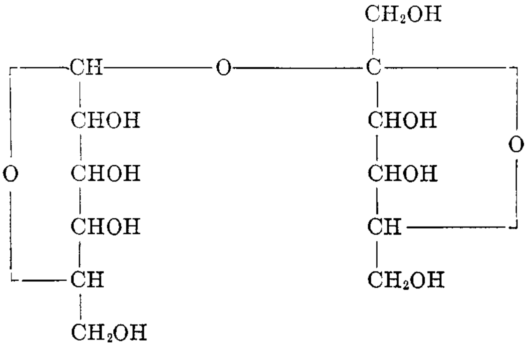
$$\begin{tikzcd}
& & \text{2:3:4:6-Tetramethyl glucose.} \\
\text{Sucrose} \arrow[r] & \text{Octamethyl sucrose} \arrow[ur] \arrow[dr] & \\
& & \text{Tetramethyl } \gamma\text{-fructose.}
\end{tikzcd}$$

and the practical issue of the constitutional study of sucrose thereafter focussed on the structure of tetramethyl γ-fructose. Haworth, who investigated this problem, claimed with a confidence which from the first seemed unwarranted, that tetramethyl γ-fructose is an amylene-oxide and that, in consequence, sucrose should be represented by:



XVI

It is regrettable that the above formula has been widely adopted, as repetition of the work has failed to confirm the results which, it may be remarked, are further vitiated by a serious theoretical mistake. The oxidation of tetramethyl γ-fructose under accurately-controlled conditions has now revealed that the compound is either a propylene-oxide or a butylene-oxide, with much indirect evidence in favour of the latter view. It follows that the formula for sucrose which can be most strongly supported is:



XVII

It may be remarked that part of the evidence leading to the above conclusion has been derived from studying those complex but interesting compounds formed by condensing glucose or fructose with acetone. This is another aspect of sugar chemistry which has been explored by the methylation process, and among the results obtained which are applicable to the problem now before us is the observation that whilst only γ-glucose condenses with acetone γ-fructose is incapable of this change which is restricted to the stable variety of the ketose.

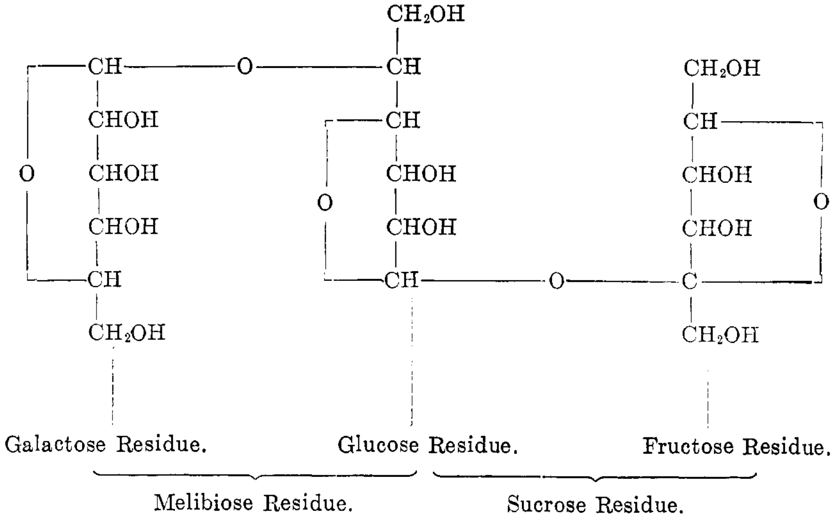
The application of the methylation process as a means of determining structure can be extended far beyond the disaccharides, and has been used to examine the structure of the trisaccharide, raffinose. Unfortunately, on this question also, I must again disagree with both the evidence and the views submitted by Haworth. It is known that raffinose can be hydrolysed in two ways which result in the formation alternatively of sucrose or of melibiose, and it follows that a satisfactory formula for the trisaccharide must incorporate the constitutions of these disaccharides. It was claimed that fully methylated raffinose gave on hydrolysis:

(*a*) 2:3:4:6-Tetramethyl galactose,

(*b*) 2:3:4-Trimethyl glucose,

(*c*) 1:3:4:6-Tetramethyl γ-fructose.

It is remarkable that this result is in agreement with formulae for melibiose and for sucrose which have been proved to be incorrect, and a scrutiny of the experimental details casts doubt on the identity of the trimethyl glucose actually isolated in the course of the work now referred to. The accepted formula for raffinose is thus incorrect in two out of the three hexose residues present, and the trisaccharide is much more probably represented by:



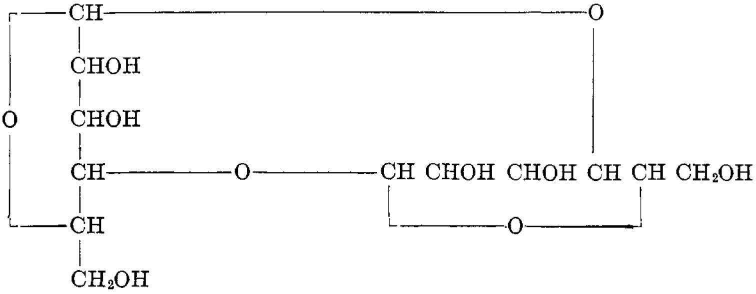
XVIII

There is, of course, one possible alternative, viz., that the attachment of galactose to glucose may be through position 4 in place of position 5 as represented above.

The desire to rectify the errors which have crept into disaccharide structure has forced me to spend a considerable time over these compounds, and we have now reached a stage when it is desirable to turn to another topic, and consider the progress in attacking the molecular structure of the polysaccharides. Speaking to this audience, I need not stress the point of the experimental obstacles which invested this section of our work with peculiar difficulty. These difficulties begin with the selection of the experimental material—if we ask ourselves the question, What are the criteria of standard specimens of cellulose or starch? we are left without an answer—and they multiply when it comes to isolating pure methylated derivatives from the colloidal systems in which they are formed. What I have called, perhaps somewhat slightingly, experimental obstacles must be regarded, however, as something more than mere inconveniences which have to be overcome, for it is only by close observation of every physical condition, as well as of every chemical property, that we can obtain a clear understanding of the polysaccharides. It would take too long to follow our various investigations in historical sequence, and I shall at once proceed to the results.

CELLULOSE

The first polysaccharide to be examined was cellulose, and it was quickly evident that the investigator, who approached this problem after experience with the subtleties of sugars proper, had to place himself in the position of a sceptical chemist. Two initial questions had to be faced: (1) is cellulose built up of glucose residues exclusively, and, (2) is cellulose based on large molecules constituted on the model of complex glucosides, or on simple anhydro-sugars highly polymerised. As a result of a series of strictly quantitative investigations, it was shown that cellulose may be converted quantitatively into glucose, and the way was thus ready to subject the polysaccharide to methylation. In this particular case, the silver oxide reaction was obviously inapplicable and, in consequence, we employed methyl sulphate and this methylation reagent has since become of general use. The introduction of the methyl groups proved to be a slow and laborious operation, but ultimately a trimethyl cellulose was obtained as a friable mass which still preserved the external appearance and fibrous nature of the parent material. Hydrolysis gave 2:3:6-trimethyl glucose as the sole product, and at once it was revealed that cellulose is molecularly constituted on an exceedingly simple plan. The polysaccharide cannot be a complex glucoside of the type first advocated by Hess, but must originate by polymerisation of a simple anhydro-*n*-glucose. There remains the question of the magnitude of *n*., and here we have, as a guide, the fact that cellulose is convertible into the disaccharide cellobiose. It follows that the simplest molecular unit for cellulose which would satisfy this condition is an anhydro-cellobiose, and such a compound, in view of the quantitative conversion of cellulose into 2:3:6-trimethyl glucose may be formulated in only one way, provided we accept the structure of cellobiose already developed:



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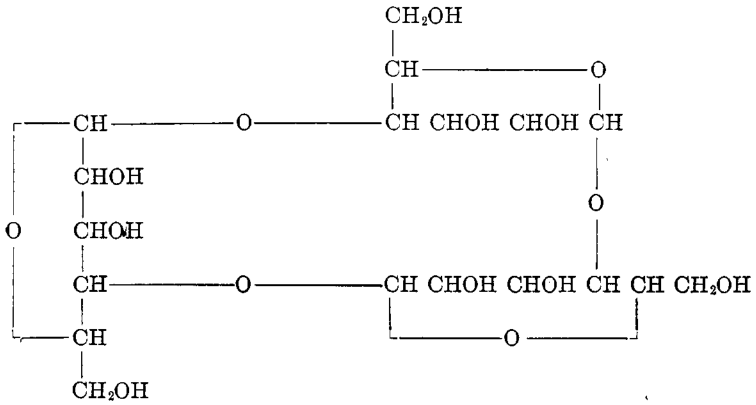
It is to be remembered, however, that, even under the most favourable conditions, the yield of cellobiose obtainable from cellulose is far short of that demanded by the above formula, so that the next possibility to be considered is that the unit may be an anhydro-trisaccharide. We are now confronted with two possible formulae, as the anhydro-cellobiose postulated above may be expanded in two ways, so as to include a third glucose residue. In discriminating between these alternatives it is undesirable to speculate too freely but, so far as our present knowledge goes, the most satisfactory formula for an anhydro-trisaccharide related to cellulose is:



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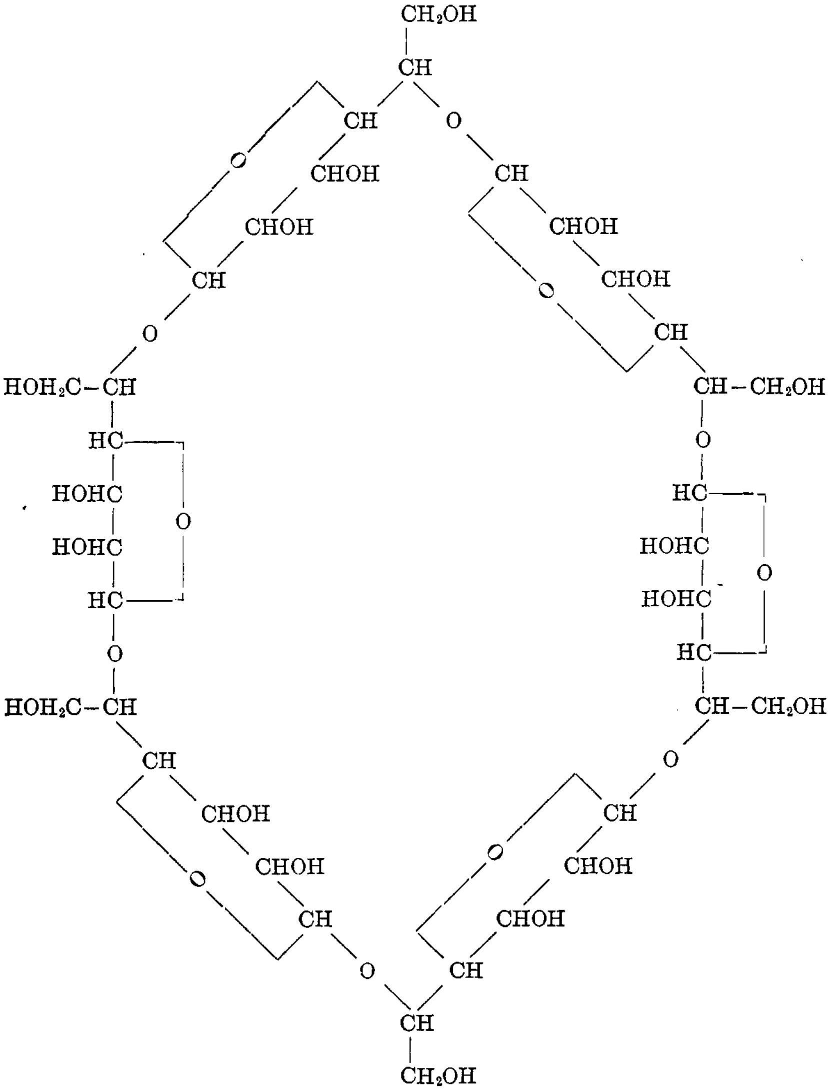
Putting this idea to the test, cellulose has been depolymerised under accurate conditions, until the simplest possible dextrins are formed, and the result then obtained is striking. The product is naturally a mixture, but the essential constituent is the dextrin $(C_6H_{10}O_5)_3$. Here again, the methylation process has been valuable, as by its aid this anhydro-trisaccharide has been converted into soluble derivatives, by means of which it was possible to establish the all-important matter of the molecular weight.

It must not be assumed, however, that cellulose is exclusively built up of anhydro-triglucose molecules. The experimental evidence so far available justifies no more than the statement that at least one-third of cotton cellulose consists of polymerised anhydro-triglucose, while the results of x-ray spectrographic analysis indicates that the C_6 residues are marshalled in even numbers, probably in groups of four or six. Taking both types of evidence into account, my view is that the celluloses may be regarded as polymerides of several basal units, in some of which an even and in others an odd number of C_6 chains are present. The contribution of the organic chemist has at least shown that these units are exclusively composed of glucose residues, that the linkage of these residues is in all cases identical, and that one of the units present is anhydro-triglucose.

STARCH AND GLYCOGEN

I have merely sketched the position reached in the study of cellulose, and must hasten to the remaining polysaccharides, starch and glycogen, which display similarly the feature of being derived from glucose. It would be attempting the impossible to enumerate, in the course of this address, the points of distinction which these compounds show relative to each other and to cellulose. Both compounds give a trimethyl derivative, differing only to a small extent in specific rotation and, in each case, subsequent hydrolysis gives 2:3:6-trimethyl glucose. Now that we know where we stand regarding maltose on methylation this result is consistent, in place of being regarded as abnormal and inexplicable.

The behaviour of starch on methylation is full of instructive features, as the process can be arrested at three definite stages, and the analytical composition of these consecutive products points strongly to the idea that there are nine, or a multiple of nine, hydroxyl groups in the unpolymerised molecule. The first compound to satisfy this condition is, of course, an anhydro-trisaccharide, while the next simplest would be the corresponding anhydro-hexa-saccharide. If we refer back for a moment to the constitution of maltose and of cellobiose you will remember that we have to make a selection from two alternatives. This necessity extends likewise to the polysaccharides which yield these disaccharides, and we have already selected one alternative formula in the case of cellulose. There remains the equally arbitrary selection of the surviving option to accommodate the case of starch, which we may thus regard as composed of glucose residues joined through positions 1 and 5. At this stage the organic chemist joins forces with the biochemist, and recognises that the action of selected enzymes on starch may give either maltose or *iso*-maltose. It is thus possible to construct a formula for starch which satisfies both chemical and biochemical considerations. This formula must contain an even number of C_6 residues, and the total number of hydroxyl groups must be a multiple of nine. These conditions are fulfilled by the following structure which is similar to that suggested by Ling.



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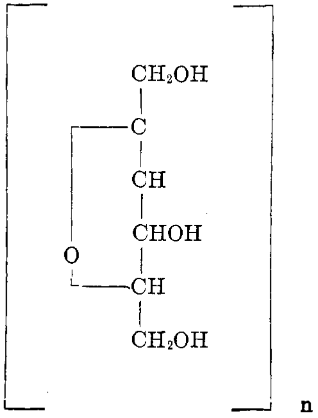
Such a formula is not founded on speculation alone. For example, it demands that out of a total of 18 hydroxyl groups twelve should be different from the remaining six, and methylation confirms this in all respects down to the actual positions occupied by these groups. Nevertheless we must be careful not to be too rigid in our views, and ought rather to remember that there is not one starch but many. We are not even justified in claiming that anhydro-hexo-glucose is the one and only molecular unit of starch, and we have no knowledge whatsoever of the different polymerides to which this unit may give rise.

The comparable researches on glycogen are concerned with minute details unsuited to a general discussion of this nature, and time permits only of brief reference to inulin, the remaining common polysaccharide. Here we are confronted with exactly the same phenomenon as we encountered in the case of sucrose, for, although inulin on hydrolysis yields laevo-rotatory fructose, this reaction provides misleading evidence as to structure. Inulin is, in fact, exclusively composed of γ-fructose residues, and treatment with acids thus initiates two consecutive reactions, the first being hydrolysis to give the unstable variety of the ketose, and the other the conversion of this sugar into the ordinary stable form. Methylation places a check on the second of these changes and, in consequence, the hydrolysis of trimethyl inulin gives a *dextro-rotatory* trimethyl γ-fructose, in which the internal oxygen ring can no longer fluctuate. This at once proves that the fructose residue in inulin is identical with that in sucrose, and we are now in a position to consider what is the simple molecule which, by polymerisation, gives the polysaccharide. The situation is a little different from that presented by cellulose and starch, as inulin differs from these polysaccharides in its failure to yield a disaccharide as the penultimate product of hydrolysis. In consequence, there is meanwhile no justification for considering any more complicated possibility than that inulin is a polymeride (or series of polymerides) of anhydro-γ-fructose, to which the following structure may be assigned:



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It is perhaps a simple statement to make, but it opens out vast possibilities for discussion, and raises the question as to the reasons underlying the fact that in the two great natural sources from which it is derived fructose exists in the γ-form.

At this stage, I must apply the closure to our review which, although lengthy, has included only the salient parts of the arguments upon which these conclusions are founded, and has left untouched the supplementary studies involved. These have not been haphazard or without design, as our investigations of such substances as the anilides, acetone derivatives of sugars, or of the phenomenon of optical activity or of polymerisation will show. We have been piecing together the framework of evidence on a systematic plan. The present is a time at which it is opportune to look into the future, as well as on the past, and to ask ourselves on what lines ought sugar chemistry to advance if it is to play its part in unfolding the mysteries of living chemistry. If I may venture to speculate, it would be to say that the constitutional study of carbohydrates will quickly reach its limit. We are fast approaching the point where we shall know what these substances are, without knowing why and how they react in Nature. The sugar chemistry of the future will, I trust, be concerned chiefly with syntheses and reactions conducted not under the artificial conditions of the organic laboratory, but within the narrow range of temperature and with the modest reagents of the living cell. It will be a chemistry controlled by severe physical restrictions, energy will be supplied by light rather than by heat, diffusion will replace filtration, polymerisation and asymmetric syntheses will be daily operations.

I began with an expression of gratitude, and, prompted by the recollection of all that has been involved in the work now laid before you, I end on the same note. Memory conjures up the loyal collaborators, drawn from all the continents, who have come to St. Andrews to give enthusiastic help. Nor do I forget what I owe to the discoveries which have proceeded from the research laboratories in this and other lands, for although under the special circumstances of my address I have confined myself largely to my own problems, I am deeply conscious of the fact that in scientific effort each man's work is but the making of a thread which goes to fashion the great fabric of truth.