THE CONSTITUTION OF POLYSACCHARIDES[1]

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In the construction of this paper I have endeavored to summarize the results of researches which have extended over twenty-one years so as to display the consecutive evidence upon which the structural representation of polysaccharides can now be based. This treatment appears necessary although no rapid survey can be made, no adequate synopsis can be given of the manifold problems of carbohydrate constitution which have already been solved or which still await adequate expression. To discuss even in broad outline the structure of a single sugar demands the consideration of a bewildering series of reactions and relationships, and to deal within the limits of a single paper with all classes of carbohydrates is to court disaster.

My excuse in attempting what I believe to be the impossible is that, to me, the carbohydrates are among the most fascinating substances which engage the attention of the chemist. To the student who brings to bear on our science the special appreciation which comes from tracing historical developments there must be abundant satisfaction in discerning how our knowledge of carbohydrates has expanded in response to the stimulus conveyed by fresh views on structure. The pioneer researches, and early explorations of the fate of carbohydrates in the living organism, must not be passed over lightly; the experimental difficulties encountered to-day serve but to remind us of the skill and patience devoted to this work. Yet, go back rather more than a century and you will find the "old masters" struggling with the sugars and giving in a few lines of print all that was then known regarding them. Bridge a gap of seventy years from their time

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[1] An essay prepared in connection with the dedication of the Sterling Chemistry Laboratory, Yale University, New Haven, Conn.

and you will find little in addition; a few more sugars had been recognized and a greater variety of derivatives had been prepared, but, in the absence of structural views, the information provided is purely descriptive and empirical. Then began that brilliant period of constitutional carbohydrate chemistry which culminated in the great awakening we owe to Emil Fischer.

It was indeed a marked advance to be able to expand the formula $C_6H_{12}O_6$ so as to display not only the significant groups but also their configuration, and thereby to account for the isomerism and mutual relationships of the simple hexoses. Almost inevitably this progressive phase was followed by a resting stage and it is not surprising that, during the past twenty-years the study of sugars has shown alternations of activity and stagnation. The present is a period of revival, and in sympathy with this growing interest, one is tempted to include many aspects of carbohydrate constitution, but, with the space at my disposal, it seems prudent to confine myself almost exclusively to problems which have engaged the attention of my co-workers and of my colleagues.

Although with the information now available an elaborate classification of carbohydrates is possible, we may, in the interests of simplicity, adhere to the present system and may divide them into three groups:

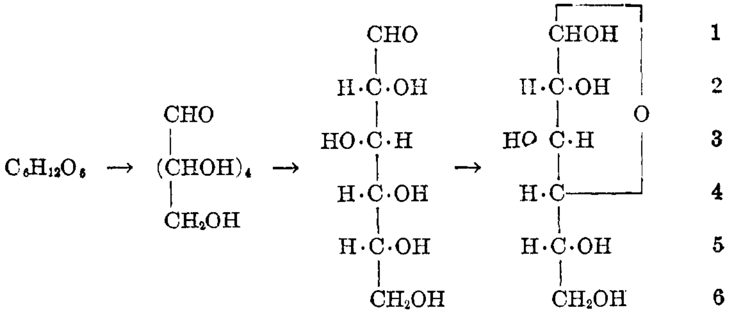
a. Reducing sugars which are unaffected by hydrolysis.

b. Glucosidic compounds which, on hydrolysis, yield at least one reducing sugar. (Di- and *n*-saccharides may be regarded as special examples of this class.)

c. Polysaccharides.

The above groups are inter-related and the constitutional problems presented by glucosides, *n*-saccharides, and polysaccharides are obviously dependent on the structure of the simple reducing sugars. Lack of knowledge of the monosaccharides is at once reflected in the complex saccharides.

In order to arrive at the fundamental aspects of carbohydrate structure we may consider the special case of glucose and trace the stages through which our present views regarding the hexose molecule have evolved. These stages are expressed in historical sequence in the scheme shown below, which displays the transition from an empirical formula to the butylene-oxide representation of α- and β-glucose now in general use.



I II III IV

The cyclic formula for reducing sugars has proved of real service and its adoption has been justified, but it is necessary to point out that, until recently, less than one half of the molecular architecture of glucose had been studied. When we remember that position 1 in the above formula plays a part in mutarotation, in the formation of glucosides, and in oxidation processes it will be agreed that this particular alcohol group has placed no obstacles in the way of thorough investigation. The properties of the group indexed as position 2 are not so well-defined but are revealed to a limited extent in the formation of osazones, while our knowledge of the group in position 6 is restricted to a few reactions such as oxidation to saccharic acid and the formation of dibromo-derivatives. Speaking generally, however, it may be claimed that the essential reactions of the three hydroxyl groups specified are already known.

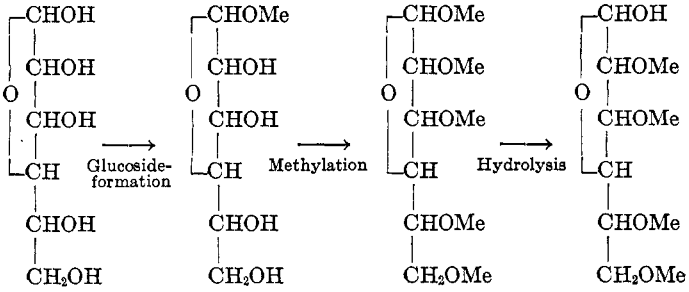
On the other hand, the asymmetric systems indexed as 3, 4, and 5 represent areas in the sugar molecule which remained almost entirely unexplored. There are good reasons for this gap in our knowledge, yet a proper understanding of the glucose molecule cannot be obtained if, as is generally the case, attention is focussed exclusively on the reducing group; each hydroxyl group must be studied, for each possesses its own particular properties and contributes to the reactions of the molecule as a whole. This consideration leads directly to another point which

I believe to be of even greater importance. It is evident that the linkage of the ring-forming oxygen atom in the molecule need not remain exclusively in one position but may connect different pairs of carbon atoms, and thus, all the groups from 1 to 6 must be regarded as variables. For example:

Position 1 may possess either the α- or β- configuration
Position 2 may be involved in an ethylene-oxide ring
Position 3 may be involved in a propylene-oxide ring
Position 4 may be involved in a butylene-oxide ring
Position 5 may be involved in an amylene-oxide ring
Position 6 may be involved in a hexylene-oxide ring

It follows, therefore, that if we include an aldehydic variety, *d*-glucose may react in any one of eleven forms or as a mixture of these isomerides. These complicated possibilities must be taken into account in speculating on the reactions of the so-called "simple" sugars, in tracing their origin, their metabolism, and their relationship to the di- and polysaccharides.

These are preliminary considerations and I turn now to an account of how the intimate structure of carbohydrates has been advanced through the agency of one particular method of enquiry, which, for reasons already given, was applied in the first instance to the monosaccharides. I refer to the process of methylation, a reaction which has been employed to introduce stable methyl groups into all the hydroxyl positions of reducing sugars or into any hydroxyl groups which remain unsubstituted and exposed in a sugar derivative. Taking an example which has been very fully studied, the sequence of typical operations leading to a fully methylated glucose may be expressed by the following scheme:



V VI VII VIII

The first stage is the formation of methylglucoside, a reaction which has the effect of protecting the reducing group, and this is followed by the introduction of methyl groups into the remaining hydroxyl positions. It is important to note that acid hydrolysis of the methylated glucoside affects the molecule only in one position, the glucosidic alkyl group being eliminated whilst the remaining methyl groups survive. In the tetramethyl glucose thus produced, four hydroxyl groups have been masked and it is thus possible to study the properties of the reducing group in the absence of many complicating factors. Extending the above principles, it will be clear that if a sugar is substituted by any group or groups capable of subsequent removal by hydrolysis, it is possible to methylate the unoccupied hydroxyl positions and ultimately to obtain definite partly methylated sugars. In these products the position of the OH groups coincides with the position in which the substituting groups were attached to the original sugar residue. For example, starting from benzylidene methylgucoside, only two hydroxyl groups are available for methylation and consequently, on completing the series of reactions, the final product obtained is a dimethyl glucose. Reference to the published papers will show that the method is of wide application and that a large variety of completely and partly methylated aldoses and ketoses have been prepared and examined.

In general, it may be said that alkylated sugars are extremely suitable for exact and critical experimental study. In many cases the compounds crystallize readily in highly characteristic forms and, in addition, the sugars or their glucosides can be effectively purified by distillation in a high vacuum. Moreover, the presence of the alkyl groups increases the solubility in organic solvents enormously, and thus the reactions of the compounds can be studied under a variety of conditions. Characterized by properties such as I have described, alkylated sugars are well-adapted as reference compounds, in that they can be isolated and identified with certainity, even when only small quantities are available. The same remark applies to the alkylated sugars which are liquids, as, in the majority of cases, they yield crystalline anilides or glucosides upon which characteristic physical constants may be determined accurately.

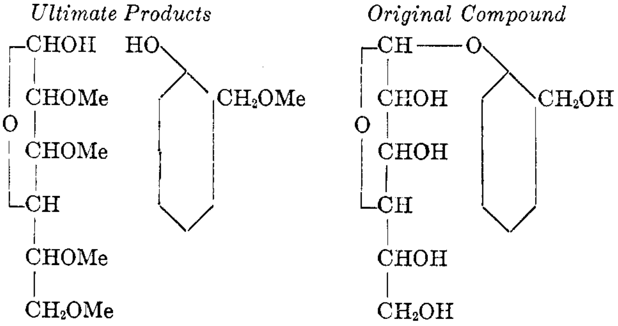
I have referred to the opportunities afforded by alkylated sugars to restrict the attack of reagents to a limited number of selected hydroxyl groups, but a more obvious application of these compounds and one which is superficially of more importance lies in the use which has been made of them to solve the structure of typical glucosides, disaccharides and polysaccharides. If we ascribe to any sugar derivative the general formula S —— G, where S is a sugar residue and G the group with which it is condensed, then methylation followed by hydrolysis will give as a minimum two products, one of which will be a methylated sugar. Determination of the number and distribution of these methyl groups in each of the cleavage products gives the complete structure of the parent compound, as the mode of attachment of the individual constituents is thereby revealed.

In the special case where the group G is also a sugar residue, the compound under consideration would be a disaccharide and the general formula may be written S —— S_1. Precisely the same structural study can evidently be applied to it with the single difference that two methylated sugars will be obtained on hydrolysis in place of one. The method is equally applicable to the general type S_n which includes polysaccharides.

This exploitation of methylated sugars in solving constitution was recognized at an early period in our work, but the policy was deliberately adopted of postponing such an extension until the standard methylated sugars required as reference compounds had been examined and definite constitutions assigned to them. Once this was done, results have come quickly and I now propose to refer to the conclusions which have been reached in a few typical cases selected from the classes into which carbohydrates are divided.

CONSTITUTION OF NATURAL GLUCOSIDES

As already explained, the application of the methylation method to α- and β-methylgulcoside results finally in the formation of one of the five different forms in which a tetramethyl glucose can exist. The sugar actually obtained is a crystalline solid, easily purified, and quickly identified by means of its physical constants. The structure assigned to it is that of formula VIII, and from what has been said, it will be evident that all carbohydrates based on the same structural model as methylglucoside will yield the same variety of tetramethyl glucose. The first case to be investigated was that of sucrose which may be regarded as a special case of a glucosidic compound. Methylation followed by hydrolysis gave crystalline tetramethyl glucose, thus solving the constitution of the disaccharide so far as the glucose component is concerned. Another example was afterwards furnished by salicin which was converted into a crystalline pentamethyl derivative and this, in turn, gave rise to equimolecular proportions of tetramethyl glucose and saligenin methyl ether. From this result, the structure of the parent glucoside is at once apparent.

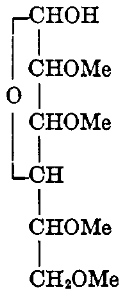


Ultimate Products *Original Compound*

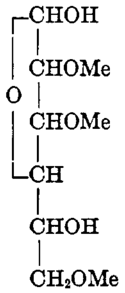
The above proof applies equally to the related glucosides helicin and populin, which must also be derived from the butylene-oxide form of glucose as they have been prepared from salicin by processes which do not disturb the glucosidic linkage. I may refer also to other results of a similar nature which have recently been obtained with important natural glucosides. It has been shown that the sugar residue in indican and arbutin has the same linkage as that in salicin, while amygdalin also falls into line and has been proved to be a glucoside of the type S —— S_1 —— G, in which the sugar residue S is similarly capable of yielding tetramethyl glucose. These are not isolated examples and it is a remarkable fact that all the natural glucosides so far examined yield the stable or butylene-oxide variety of tetramethyl glucose. This result may be translated into the statement that although glucose can and does exist in a highly reactive form, this variety does not appear to play a permanent part in the formation of glucosides in Nature. Reference will be made later to artificial glucosides derived from an unstable modification of glucose, but at this stage it may be stated that such compounds show little tendency, in the absence of hydrolytic conditions, to revert to the more stable isomerides by alteration of the oxygen ring. This change seems in fact to be dependent on the presence of the reducing group, and the combined evidence indicates that it is the stable form of glucose which is utilized by plants in the formation of natural glucosides. Some indications exist that the glucosidic components of plant pigments are not invariably derived from a single variety of glucose, but our work in this direction is not sufficiently advanced to permit of a final decision being made.

CONSTITUTION OF DISACCHARIDES

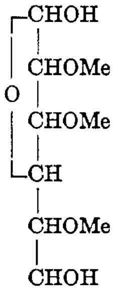
Five methylated hexoses, functioning in the capacity of reference compounds, have served to determine the essential constitution of the most important disaccharides and polysaccharides. The hexoses in question are: tetramethyl glucose, two isomeric forms of trimethyl glucose, tetramethyl γ-fructose, and tetramethyl galactose.


2:3:5:6-Tetramethyl glucose or galactose

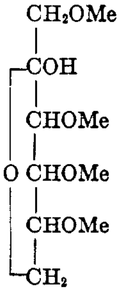
IX


2:3:6-Trimethyl-glucose

X


2:3:5-Trimethyl glucose

XI


Tetramethyl-γ-fructose

XII

Of these, 2:3:5:6-tetramethyl glucose has proved of greatest service, and it is fortunate that the compound is the most readily identified of all methylated sugars.

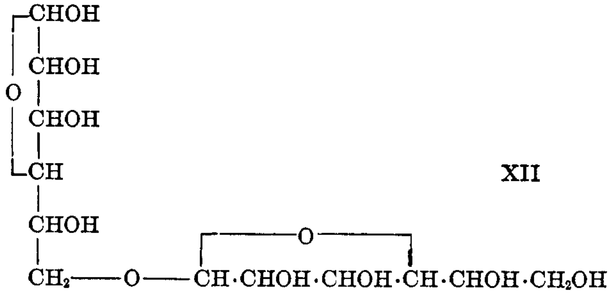
In the experimental treatment of reducing disaccharides the procedure adopted with monosaccharides is followed in that a glucosidic methyl group is introduced before the methylation of the sugar chain is carried out. By a simple but extremely effective method, these consecutive reactions are conducted in one operation and in this way a number of fully methylated disaccharides have been obtained. The hydrolysis of these compounds has given results which, as in the examples already quoted, are decisive in displaying the manner in which the hexose components are united. As the principles involved have already been explained the structural studies of typical disaccharides can be readily synopsized.

Maltose

The essential steps required in this particular investigation are shown below:

$$\text{Maltose} \rightarrow \begin{matrix}\text{Heptamethyl}\\ \text{methylmaltoside}\end{matrix} \rightarrow \begin{cases}\text{2:3:5:6-Tetramethyl glucose}\ldots\ldots\ldots\ldots\text{IX}\\ \text{2:3:5-Trimethyl glucose}\ldots\ldots\ldots\ldots\ldots\text{XI}\end{cases}$$

tilizing the structures for IX and XI, the coupling of the Uucose residues in maltose is shown to be:


XII

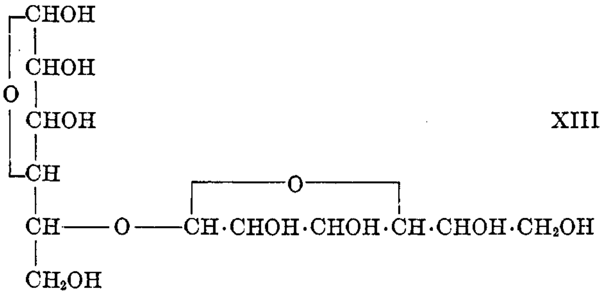
The formula thus established finds application, as will be described later, in deducing the constitution of the starch molecule.

Cellobiose

An entirely different mode of combining two hexose residues is displayed by cellobiose, as one glucose molecule is attached through its reducing group to the 5 position of the second glucose molecule. This is shown from the sequence of changes:

$$\text{Cellobiose} \rightarrow \begin{matrix}\text{Heptamethyl}\\ \text{methylcellobioside}\end{matrix} \rightarrow \begin{cases}\text{2:3:5:6-Tetramethyl glucose}\ldots\ldots\ldots\text{IX}\\ \text{2:3:6-Trimethyl glucose}\ldots\ldots\ldots\ldots\ldots\text{X}\end{cases}$$

From these results it follows that cellobiose should be represented by the structure:



XIII

Apparently the attachment of the hexose molecules depicted in the above formula is characteristic only of natural carbohydrates, as the same coupling is present in cellulose, in starch, and in glycogen but not in synthetic dextrins. Meanwhile it is of interest to find that precisely the same condition exists in the disaccharide lactose.

Lactose

The mutual relationships of the glucose and galactose residues in lactose are at once evident from the experimental scheme:

$$\text{Lactose} \rightarrow \begin{matrix}\text{Heptamethyl}\\ \text{methyllactoside}\end{matrix} \rightarrow \begin{cases}\text{2:3:5:6-Tetramethyl galactose}\ldots\ldots\ldots\ldots\text{IX}\\ \text{2:3:6-Trimethyl glucose}\ldots\ldots\ldots\ldots\ldots\ldots\text{X}\end{cases}$$

Considering the origin of lactose, it is important to find that the galactose component belongs to the stable type and that the reactive, or γ-form, is definitely absent. This point was readily established, as although tetramethyl galactose is a liquid, it was identified by conversion into the well-defined crystalline anilide in yields which show that no other isomeride was present.

Taking into consideration the mutual relationships of the compound sugars, the constitution of other reducing disaccharides may be deduced from the results already obtained experimentally, but meanwhile the complicated case presented by sucrose may be discussed.

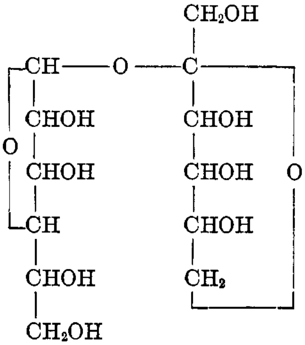
Sucrose

So far as two factors are concerned, research on sucrose proved to be comparatively simple. In the absence of a reducing group the actual methylation presented few difficulties and tetramethyl glucose, which is one of the scission products obtained from octamethyl sucrose, crystallizes readily. Closer inspection of the practical details will, however, reveal some of the obstacles encountered.

$$\text{Sucrose} \longrightarrow \begin{matrix}\text{Octamethyl}\\ \text{sucrose}\end{matrix} \longrightarrow \begin{cases} \text{2:3:5:6-Tetramethyl glucose} \ldots\ldots\ldots\ldots\ldots\ldots \text{IX} \\ \text{a Tetramethyl fructose} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{XII} \end{cases}$$

The fructose derivative was a liquid and its separation by distillation proved to be tedious and imperfect. This was overcome by replacing octamethyl sucrose by the corresponding heptamethyl derivative, but another difficulty was then encountered, as the constitution of the tetramethyl fructose was unknown. The compound was moreover abnormal, being dextro- in place of laevo-rotatory, and although earlier investigations had already revealed this irregularity, prolonged research has been necessary in order to ascribe a formula to the reference sugar.

It is not known that the compound belongs to the type generally known as "γ-sugars," and I hope to refer to these curious isomerides at a later stage. Meanwhile, it may be stated that the normal laevo-rotatory form of fructose has been definitely proved to be a butylene-oxide, and that in the tetramethyl fructose now under consideration the internal oxygen ring is displaced to one of the alternative positions. The first suggestion that an ethylene-oxide ring is present, is no longer tenable, and a former colleague provides experimental evidence in favor of an amylene-oxide structure. According to this view the formula for sucrose becomes:



XIV

It may be mentioned that both the dimethyl and the trimethyl fructose obtained from inulin are likewise γ-sugars, and contain the same internal ring which is present in the ketose fragment of sucrose. The examination of these sugars, which is in progress, will throw further light on the structure of sucrose.

From what has been said, it will be admitted that a new complication has been introduced into studies on the inversion of sucrose, as the initial condition of one of the hydrolysis products is transient. The disaccharide thus furnishes an interesting case where methylation reveals a feature of molecular structure which other processes fail to detect.

POLYSACCHARIDES

Although doubtless any reducing sugar is capable of yielding a corresponding polysaccharide, authentic examples of the latter class of compound are not numerous and, up to the present time, our attention has been restricted to the four most important representatives: cellulose, starch, glycogen and inulin. It is perhaps advisable to express the opinion that we are scarcely justified in claiming that the names used above refer to individual compounds and, strictly speaking, we should meanwhile regard them as applicable to groups of close-related compounds.

Systematic study of the polysaccharides on constitutional lines is naturally more difficult than that of the related sugars. The absence of crystalline structure, the inconvenient solubilities involved, and the uncertainty regarding the uniformity of the material under examination are disadvantages, the full measure of which quickly becomes apparent in the experimental treatment of the compounds. These obstacles have not been allowed by chemists to stand in the way of investigating polysaccharides, which have been examined with commendable patience, yet it must be admitted that the reactions available for elucidating constitution are few in number and are difficult to interpret. Above all, the lack of precise information leading even approximately to the molecular weight of members of the group has proved a serious disadvantage. Two fundamental points, however, have long been recognized. The polysaccharides as a class possess the formula $(C_6H_{10}O_5)_x$ and are apparently polymerides of definite molecular units. Although little information can be gleaned from an empirical formula of this nature, the situation becomes clearer when it is remembered that, on complete hydrolysis, each polysaccharide is converted into a monosaccharide. Familiar examples are:

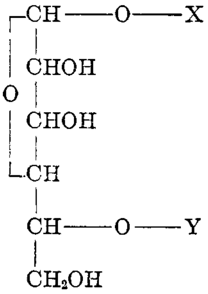
Cellulose ⟶ Glucose
Starch ⟶ Glucose
Glycogen ⟶ Glucose
Inulin ⟶ Fructose

The evidence afforded by hydrolysis is important but, on occasions, may be utterly misleading, and consideration of one example will give an indication of the pit-falls which await the investigator in this field. The fact that inulin is converted by the action of dilute acids into the ordinary laevo-rotatory variety of fructose led very naturally to the idea that the polysaccharide is derived from this form of the sugar. Such is not the case, and it is now certain that the fructose molecule liberated when inulin is decomposed is a dextro-rotatory ketose, which reverts at once to the stable laevo-rotatory variety. An extreme case, parallel with that of sucrose, has been quoted, but it serves to illustrate the caution which must be exercised in work of this description. Failure to appreciate the complex isomerism of which the monosaccharides are capable has led in other cases to hasty speculation.

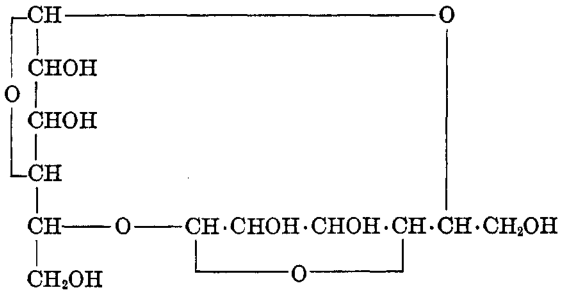
Although, from the structural point of view, inulin is the most unique and, in many respects the most interesting of the polysaccharides, it is perhaps advisable to deal, in the first instance, with members of the class which are related to glucose. I turn, therefore, to the case presented by cellulose.

Cellulose

In a paper recently presented before the Chemical Society of London, results are described from which the constitution of the molecular unit of cotton cellulose may be deduced. To avoid repetition, I shall omit a detailed discussion and confine myself strictly to a synopsis of the essential evidence. Cotton cellulose has been converted into α-methylglucoside in yields which are practically quantitative, so that there can be little reason to doubt that polysaccharide is composed entirely of glucose units. This result, of course, gives no clue as to the linkage of these glucose residues, but the information has been supplied by a parallel research on fully methylated cellulose. The alkylation proceeds readily up to the dimethyl stage, but thereafter the methoxyl content rises very slowly, and it has been a matter of some difficulty to obtain a trimethyl cellulose and to prove that this product represents the experimental limit of the substitution. Now, it has been known for some years that when dimethyl cellulose is hydrolyzed there results a complex mixture of methylated sugars, one component being 2:3:6-trimethyl glucose. This result, significant in itself, justified only one step being taken in ascribing a formula to cellulose which, in consequence, might be represented by the expression:

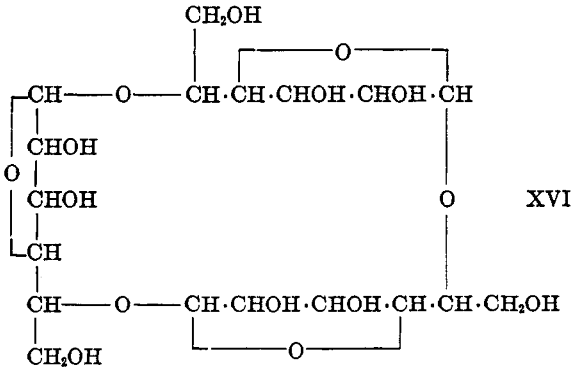


Beyond the fact that they must consist of some form of glucose residues, the nature of the groups X and Y remained quite unknown until the fully methylated cellulose I have mentioned was available for hydrolysis. In addition a further complication was introduced (and the point is somewhat elusive) through lack of any information as to how the hydroxyl groups in cellulose are distributed between the different C_6 units. Applying the considerations already discussed, reflection will show that according to the manner in which the hydroxyl groups are shared by each C_6 component, a trimethyl cellulose may undergo hydrolysis in four different ways and that, as a maximum, 49 methylated sugars may then be produced. These formidable possibilities are dealt with in the paper referred to, but the result actually obtained was the simplest of all the alternatives. Trimethyl cellulose was converted quantitatively into 2:3:6-trimethyl methylglucoside and this, in turn, yielded the corresponding sugar, unmixed with any isomeride or with any other derivative of glucose. A striking simplicity is thus established for the cellulose molecule, as the unknown fractions X and Y are shown to be identical with each other and with the nucleus glucose residue. In order to expand this result into a molecular structure, it is necessary to know how many of these glucose residues go to form the unit. The question is largely solved when the constitution of cellobiose is taken into account, as good grounds exist for claiming that this sugar is a definite fraction of the cellulose complex. The simplest expression of the cellulose molecule therefore consists of two anhydroglucose residues connected as shown in formula XVI:



XVI

Further expansion of the formula, is however, necessary to accommodate the fact that the yield of cellobiose from cellulose does not exceed 50 per cent and this important condition is satisfied by including three glucose residues in the molecule. There are four different ways of building up such a structure, but three of the formulae thus obtained imply that cellulose should be convertible into other disaccharides in addition to cellobiose. Experimental evidence is, on the whole, against this view and, as the remaining alternative is free from such an objection, it is meanwhile selected as the most appropriate.



XVII

The essential reactions of cellulose are satisfied by the above formula, but in two respects, the results furnished by the methylation process fail to complete the architectural scheme of the cellulose complex. No indication is given of the α- or β-configuration of the glucose components, and no idea is conveyed of the mechanism or extent of the polymerization undergone by the simple molecule. We realize, however, that our work is not at an end and it may be stated that, by the degradation of esparto cellulose, a compound corresponding to the molecular unit postulated above has already been isolated. Now that the outlines of the cellulose molecule have been defined, many further developments will be apparent and we are engaged in extending our researches to various forms of modified celluloses.

Starch

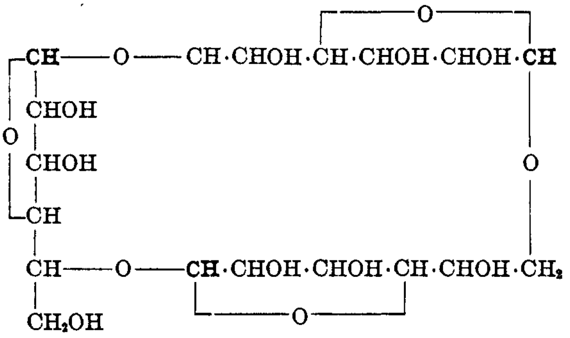
Turning to the problem of the constitution of starch, very much the same difficulties emerge as have been referred to in the case of cellulose and, viewed superficially, the two polysaccharides have much in common. Like cellulose, starch is composed entirely of glucose residues and contains three hydroxyl groups for each C_6 unit present in the molecule. It does not follow, although this is almost invariably assumed, that each glucose residue contains three substituted hydroxyl groups, or that the distribution of the three groups is uniform. The primary reaction of starch which, so far as we can tell, must be accommodated in a structural formula is the production of the disaccharide maltose by the action of diastase. Starch is also distinguished sharply from cellulose in being more readily hydrolyzed by acids and this at once points either to

1. A different type of polymerization
2. A different mode of attachment between the individual sugar residues.
3. A variation in the type of the glucose units

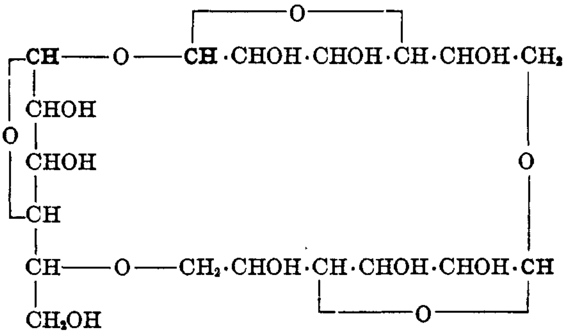
In other words, part of the starch molecule might conceivably be related to γ-glucose, but this possibility can now be definitely excluded from the results furnished by methylation. These results are in themselves simple, but few reactions impose greater demands on the experimenter than the alkylation of starch.

As a rule, the procedure in methylating compounds of this nature is to use, in the first instance, methyl sulphate as the reagent and, when the methylation has proceeded far enough to render the product soluble in organic solvents, to complete the substitution by means of the silver oxide reaction. In the case of starch, there is clear evidence that these alternative reagents act in a different manner, owing to the preferential attack of silver oxide and methyl iodide on one particular hydroxyl group. The methylation can, in fact, be arrested definitely when a dimethyl starch is the sole product (OMe = 32 per cent), but by varying the procedure, the methoxyl content can be gradually increased until it reaches the value 37 per cent. It should be stated that this is not an absolute maximum, but represents a second definite stage beyond which further methylation becomes a matter of increasing difficulty. The lower value for methoxyl (32 per cent) corresponds, as stated, to a dimethyl starch, and no definite structural evidence is forthcoming from the hydrolysis of this compound. On the other hand, the higher methoxyl content (37 per cent) agrees with the theoretical amount required for an anhydrotrisaccharide in which three methyl groups are attached to one glucose residue, while four such groups are shared by the two remaining glucose residues. The same result has been obtained consistently in different preparations, and ultimate analysis has confirmed the composition. Hydrolysis of this methylated starch shows that this is not a coincidence. The sugars thus produced were isolated in the form of the corresponding methylglucosides, and separated by vacuum distillation. Two products were in this way obtained, one being a trimethyl methylglucoside, and the other a dimethyl methylglucoside. It is significant that these products were formed in the molecular ratio of 1:2. On isolating the respective sugars from the compounds, a totally unexpected result was obtained, as 2:3:6-trimethyl glucose was produced from the trimethyl methylglucoside. As will be seen from inspection of formula X we are forced to the conclusion that in starch one glucose component is linked in a manner which is duplicated in cellulose, cellobiose, and in lactose but is absent in maltose. Combining these factors, it follows that, as a minimum, the starch molecule must contain three glucose units arranged in such a manner that one pair displays the coupling present in maltose while another pair reproduces the linkage characteristic of cellulose. The results summarized in the scheme given below indicate the conditions which must be satisfied, and these are fulfilled by any ony of four formulae.

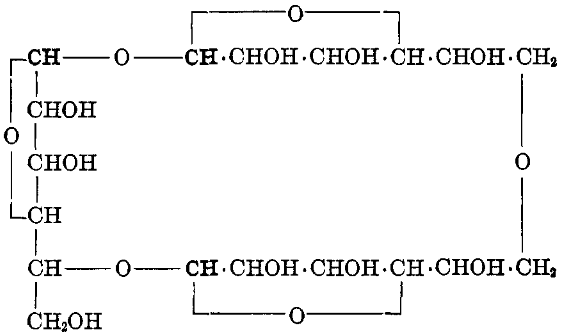
Starch ↗	Maltose →	2:3:5:6-Tetramethyl glucose	1 mol.
		2:3:5-Trimethyl glucose	1 mol.
Starch ↘	Methylated starch →	2:3:6-Trimethyl glucose	1 mol.
		Dimethyl glucose	2 mols.



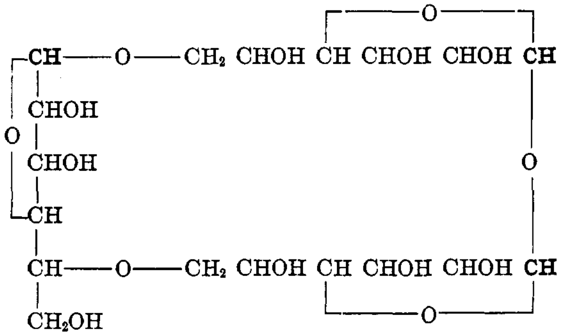
XVIII



XIX



XX



XXI

(Letters in bold face type designate the potential reducing groups)

In reviewing the four possibilities, there is no diagnostic reaction which will enable a final decision to be made between them but the formulae differ in one important respect. Maltose may be produced in one way only from either formulae XIX, XX, and XXI and in two ways from the remaining alternative which is thus preferred until the completion of work now in progress. The objections which may be offered against the adoption of such a formula have been discussed elsewhere, and the view now expressed receives strong support from the fact that very similar results have been obtained with glycogen.

Glycogen

In general it may be said that the methylation of glycogen displays precisely the same features as were encountered in the case of starch. The reaction shows a distinct tendency to cease at a stage where a dimethyl glycogen is the essential product, but frequent repetition of the alkylation occasions a slow increase in the methoxyl content, which has now been raised to a value close to that required for a trimethyl derivative. In a number of respects, however, minor differences are apparent between methylated starch and methylated glycogen and these suggest that although a close parallel exists between the respective molecular units, they are unlikely to prove identical.

One highly important factor has already been established with certainty, and that is the formation of crystalline 2:3:6-trimethyl glucose by the hydrolysis of a methylated glycogen containing 37 per cent of methoxyl. The reaction which gave this result proceeds in exactly the same way as the hydrolysis of methylated starch and points to the idea that an anhydro-trisaccharide represents the glycogen molecule. Consequently both starch and glycogen resemble each other and also cellulose in possessing the unit shown in formula X. Beyond this, it is impossible to speak with certainty at the present time as the constitution of the other hydrolysis products obtained from methylated glycogen has not been fully developed, but the evidence so far accumulated indicates that one of the formulae XVIII, XIX, XX, and XXI, represents the molecular unit of glycogen.

Taking a general view of the three polysaccharides related to glucose, a number of interesting points emerge. The first undoubtedly is their surprising simplicity. In the production of these compounds in Nature complications of structure have been avoided, and although in the end there are wide differences between the polymerized aggregates to which we apply the names cellulose, starch and glycogen, the inner molecular structure is remarkably alike. Apparently the contrasting properties of these polysaccharides are dependent chiefly on the extent and, more particularly, on the mechanism of the polymerization undergone by the molecules, and we know little or nothing concerning these factors. Analogy is dangerous, but as we are dealing with molecular architecture, I may remind you that many different buildings, varying in design and function, may be erected with bricks of identical material and mould. The analogy may serve to reveal my opinion of the vast problems which have still to be solved before we can trace how, and why, an anhydro-trisaccharide molecule can take up its place as part of a cellulose fibre or of a starch grain.

A second generalization is that polysaccharides are not complex glucosides in which the groups of one glucose residue are substituted by chains of other glucose residues. Two synthetic dextrins have been shown to conform to this model, but no evidence is forthcoming that any natural polysaccharide is constituted in such a way. But the most remarkable feature in cellulose, starch and glycogen is the absence of any sugar residue possessing the structure of γ-glucose, and on first inspection, this may be regarded as somewhat damaging to the theory that the reactive type of this sugar plays a part in natural syntheses. On the other hand, a greater importance must hereafter be attached to position 5 in the glucose molecule, as it functions in the coupling of the hexose residues in five highly important saccharides. In submitting these formulae, it is perhaps necessary to sound a note of caution, as the double assumption is made that maltose is an integral part of starch, and similarly that the cellobiose unit is present in cellulose. There seems no reason to doubt these conclusions and they are generally accepted, but they have not been proved.

Issues which are of special interest to the physiologist are raised in the relationship now established between starch and cellulose on the one hand, and between starch and lactose on the other, as one molecular fragment is common to all the compounds. A similar statement may be made regarding sucrose and inulin, as will be evident from the consideration of this polysaccharide.

Inulin

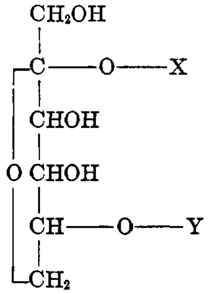
A common view is that inulin is the direct analogue of starch from which it differs in being based on fructose in place of glucose. The distinction is, however, more deep-seated than the above statement implies, and inulin occupies a position which is unique among natural carbohydrates. It is the only compound, so far as known, which is built up of hexose units belonging exclusively to the type known as "γ-sugars." This feature of the polysaccharide has already been referred to, and a diagrammatic scheme will show the succession of reactions which lead to this conclusion.

$$\begin{tikzcd}
\text{Inulin} \arrow[r] \arrow[d] & \underset{\text{(laevo)}}{\text{Fructose}} \arrow[r] & \underset{\text{(laevo)}}{\text{1:3:4:6-Tetramethyl fructose}} \text{ .... A} \\
\underset{\text{inulin}}{\text{Trimethyl}} \arrow[r] & \underset{\text{(dextro)}}{\text{Trimethyl fructose}} \arrow[r] & \underset{\text{(dextro)}}{\text{Tetramethyl } \gamma\text{-fructose}} \text{ .... B}
\end{tikzcd}$$

The ultimate products A and B are definitely isomeric, and a fundamental change in structure must therefore take place during one of the reactions involved. On the basis of evidence which, if complicated, is nevertheless conclusive, it has been shown that it is the conversion of inulin into laevo-rotatory fructose which is at fault. During this change the internal ring in the sugar assumes the stable butylene-oxide position. This is not present in the original polysaccharide, but no such intramolecular change from the unstable to the stable type is possible in the parallel hydrolysis of trimethyl inulin, as the hydroxyl group necessary for the alternation is substituted.

Our earlier work on inulin established several points of primary importance. All the fructose residues were proved to conform the γ-type, and moreover, each C_6 unit was shown to contain three hydroxyl groups. One important piece of structural evidence remained undetermined but this can now be supplied.

If the hydroxyl groups in inulin are similarly distributed among all the C_6 chains then the trimethyl γ-fructose obtained from trimethyl inulin must be a single chemical individual. Conversely, in the event of the hydroxyl groups being unsymmetrically arranged, then a mixture of the four possible trimethyl γ-fructoses would result. To obtain the necessary evidence has been difficult, and has demanded the preparation of large quantities of trimethyl γ-fructose which, unfortunately, is a viscous uncrystallizable syrup. The liquid has been shown to be homogeneous in every respect, and the complete symmetry of the inulin molecule is thus established. It is, on paper, a simple matter to utilize these results so as to construct a molecular formula for inulin, but some doubt still remains as to the constitution of trimethyl γ-fructose. The compound has the same internal oxygen ring which is present in the ketose component of sucrose, but the position of the unsubstituted hydroxyl group is not yet determined with certainty. Until this has been done, it is premature to ascribe a complete structural formula to inulin but the most probable structure is



XXII

the same unit being represented at XY or at X and Y.

It is my belief that the key to the mechanism of Nature's method of building up polysaccharides from primitive molecules lies in the comparative study of starch and inulin. These natural processes, must at some stage, involve the formation of a compound which by simple intramolecular change gives rise, according to the conditions, to either glucose or fructose. The liberation of one of these sugars leads ultimately to starch or inulin respectively, while the simultaneous formation of the hexoses would result in the formation of sucrose. This suggestion is not at variance with the order in which sucrose and starch make their appearance in the plant, provided it is established that the ketose molecule can be rapidly formed and speedily removed by condensation. Such a condition is not met by the reactions of the ordinary variety of fructose, but is fulfilled by the highly reactive modification of the sugar which is included under the expression "γ-fructose." This brings me to the consideration of a novel aspect of carbohydrate chemistry.

γ-SUGARS

In the preceding sections, I have been under the necessity from time to time of referring in passing to γ-sugars and it seems expedient, in closing, to summarize our knowledge regarding these important variations in sugar molecules. This is desirable, as hitherto no attempt has been made to compile a general survey of what is known regarding γ-sugars and, as considerable interest is taken in these compounds, I begin with the statement that the name applied to them is not descriptive but is, in a sense, accidental.

As is well-known, the best-defined reducing sugars can generally be obtained in two isomeric forms which display muta-rotation in opposite directions, and this is attributed to the rearrangement of the groups in the reducing position. The expressions α- and β- were applied to designate these isomerides, and the two muta-rotatory forms of glucose serve as standard examples in illustration. Now this isomerism has been proved to be in no way dependent on the position of the internal oxygen ring in the sugar since both α- and β-glucose have been correlated with the butylene-oxide form of tetramethyl glucose. The discovery that another variety of glucose exists, in which the oxygen ring is differently attached, could not be accommodated by the nomenclature then in use and the expression "γ-glucose" was applied to it. The name "γ-sugar" has therefore a very general significance, and is used to include all forms in which the oxygen ring is displaced from the normal stable position. This is not entirely satisfactory, but a more exact nomenclature cannot be applied until the complete structure of each of these sugars has been established. Meanwhile it may prevent confusion if it is pointed out that γ-sugars, retaining as they do a reducing group, can exist in α- and β-modifications, and the same remark applies to their glucosides.

Up to the present it has been ascertained that glucose, galactose, mannose, rhamnose and fructose are all capable of existing in γ-forms and there seems to reason to doubt that the property is general. In no case, however, has an unsubstituted γ-sugar been isolated, although we recognize their transient existence, and can in some measure study their properties by examining their methylated derivatives. The presence of the methyl groups in these derivatives prevents any alteration in the oxygen ring, and thus, the constitutional type is preserved.

Before summarizing the chemistry of γ-sugars, it may be mentioned that their discovery did not originate in any single dramatic observation, but that the evidence accumulated gradually. One of my laboratory note-books, describing work carried out exactly twenty years ago, gives an account of the methylation of sucrose, and the separation of the sugars thereafter formed on hydrolysis. Crystalline tetramethyl glucose was isolated and, in addition, the corresponding fructose derivative was obtained by means of condensation in the cold with methyl alcohol, followed by the usual fractional distillation and hydrolysis. The complete analysis of the product was carried out, its composition was ascertained to be that of a tetramethyl fructose, and its specific rotation was shown to be of the order +27°. The entry in the book states that the work was at this stage suspended, there being no adequate explanation as to why a methylated fructose should be dextro-rotatory. The compound was, in fact, pure tetramethyl γ-fructose, but many consecutive researches were necessary before the discovery could be interpreted. A subsequent paper on the methylation of fructose added materially to the evidence that the ketose can react in a dextro-rotatory form, and about the same time, a result of equal significance was forthcoming. A laevo-rotatory trimethyl glucose was obtained from glucose monoacetone and, in investigating the origin of this compound, a new laevo-rotatory form of tetramethyl methylglucoside was isolated, *thus showing that not only can d-fructose react in a dextro-form but d-glucose can exist in a laevo-rotatory variety.*

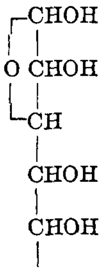
At this stage Fischer published his paper on γ-methylglucoside which was quickly followed by our account of the isolation and properties of tetramethyl γ-glucose. In view of this theoretical development, the way was now clear to resume the study of sucrose and to expand the scope of our work.

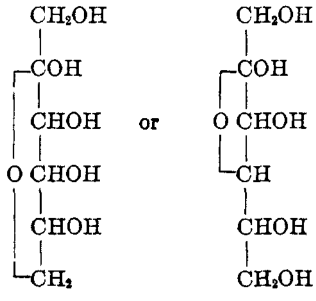
For reasons already stated, the properties of γ-sugars have to be inferred from those of their methylated derivatives and, so far, the following examples together with the corresponding "methylglucosides" are available for study:

Tetramethyl	γ-glucose	(laevo)
Trimethyl	γ-glucose	(leavo)
Tetramethyl	γ-galactose	(laevo)
Tetramethyl	γ-mannose	(laevo)
Tetramethyl	γ-fructose	(dextro)
Trimethyl	γ-fructose	(dextro)

In reviewing their reactions, one is struck by the close resemblance shown to natural processes. For example, the compounds undergo oxidation with extreme ease, being affected by mild oxidizing agents, and in illustration it may be mentioned that trimethyl γ-glucose reduces Fehling's solution instantaneously in the cold. At ordinary temperatures, also, they are converted into glucosides, at a speed which is remarkable, especially when it is remembered that the formation of α-methylglucoside requires a treatment of approximately sixty hours at 100°. In at least one case (tetramethyl γ-galactose) a reducing sugar of the γ-type is known to pass spontaneously by auto-condensation into a non-reducing disaccharide. These striking properties are far removed from those of a normal sugar.

Great difficulties have been encountered in attempts to solve the constitution of γ-sugars and experimental work has been largely confined to tetramethyl γ-glucose, trimethyl γ-fructose and tetramethyl γ-fructose. In the case of the glucose derivative, the first opinion formed was that an ethylene oxide ring was present, but this view has been modified in favor of the propylene-oxide structure. Similarly, an amylene-oxide constitution is at present supported for tetramethyl γ-fructose so that, meanwhile, the parent sugars may be represented as:


γ-Glucose



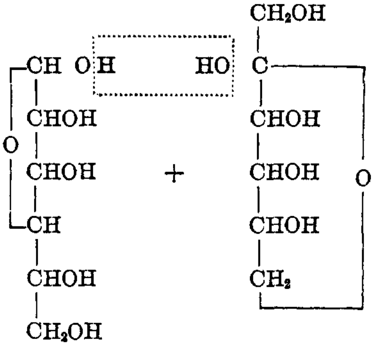
γ-Fructose

It is already evident that the butylene-oxide structure cannot be assumed for any sugar even in its most stable form. Thus crystalline xylose, which displays none of the properties of a γ-sugar, has recently been shown to be an amylene-oxide and this respect at least resembles γ-fructose.

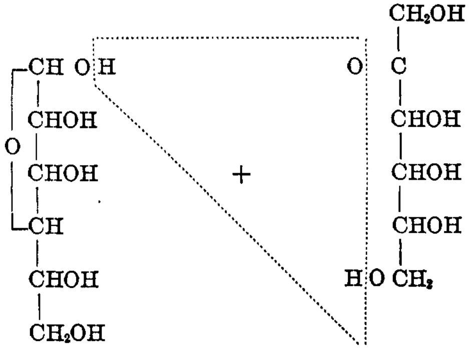
I admit that speculation in the absence of experiment is best avoided in the carbohydrates, but in the course of investigating many varieties of γ-compounds, I found it impossible to reject the idea that the ordinary, crystalline, comparatively stable sugars of the laboratory are not the forms which are primarily elaborated by the plant or disrupted by the animal. The fructose which we store in the chemical museum, and produce as a lecture specimen, is not fructose as it exists in combination in sucrose or in inulin.

The idea conveyed above is that the natural processes leading to the formation of sugars and their derivatives, as also the ultimate utilization of carbohydrates by the animal organism involve as intermediate stages the transient formation of γ-forms. The evidence may not be particularly convincing save to those who have handled all types of carbohydrates at the working bench, but it is certainly the case that we have fleeting glimpses of extraordinarily reactive sugars which, in the process of artificial isolation from either plant or animal sources, undergo rearrangement to the sugars as we know them. This is not inconsistent with the results obtained in either hydrolysis or synthesis effected by enzymes. If this suggestion is substantiated, it may have far-reaching effects in studying physiological processes, and in the treatment of conditions such as diabetes in which the metabolism of sugars is abnormal. Research designed to test this idea was commenced three years ago, and the first tentative steps have been taken to ascertain if living tissue promotes the formation of γ-glucose. The evidence obtained is encouraging, but further progress is hampered by the inaccessibility of γ-sugars, and the lack of trustworthy methods of identifying them. When it is remembered that γ-glucose and γ-fructose revert instantaneously to their respective stable forms in the presence of 0.1 per cent of hydrochloric acid, it will be agreed that there is little prospect of detecting these fugitive isomerides by ordinary processes. Analytical methods are of no avail, and polarimetric evidence alone is insufficient and often misleading. No doubt these difficulties will be surmounted in time, but only when the detailed chemistry of γ-sugars has been advanced on systematic lines, and when knowledge exceeds impatience.

Having in a sense created γ-sugars for you, it may appear contradictory if I say that I have some doubts as to whether these substances are, after all, chemical individuals. They may only represent a condition of the sugar molecule which originates in the mechanism whereby a sugar forms certain of its derivatives. To make the point clear by means of an example, we need not assume that in the formation of sucrose a molecule of normal glucose condenses with a molecule of γ-fructose in such a manner that each loses a hydroxyl group.



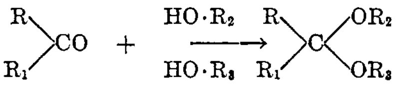
I suggest that γ-fructose may have no real existence, and that the condensation may be equally well explained on the assumption that fructose functions as a ketone. In such a case, two hydroxyl groups become involved, and although one of these must be the reducing group of the aldose molecule, there is no reason, once the reaction is initiated, why the ketose itself should not furnish the second group:



An amylene-oxide ring is thus formed in the ketose component and here we see *the production of a γ-residue without the previous existence of a γ-sugar.* Similar considerations apply to the formation of γ-glucose derivatives which may be formed in condensation reactions in which glucose reacts as an aldehyde. These suggestions are not without the support afforded by numerous analysis, and meanwhile the whole subject is a maze of possibilities, and enlightenment is still remote.

For example, if the question is raised as to why sucrose is so remarkably unstable towards hydrolysts the existence of an amylene-oxide ring in the fructose component provides no answer. Methyl xyloside is likewise an amylene-oxide, yet the compound resists the effect of dilute acids while β-glucosan which contains a hexylene-oxide ring is notably stable. Again, fructose mono- and diacetones both undergo hydrolysis with a facility which resembles that displayed by sucrose but nevertheless both of these compounds are based on the stable type of fructose in which a butylene-oxide ring is present. These apparently contradictory results reveal that the special instability of γ-derivative does not depend primarily on the presence of any particular internal ring in the sugar.

On the other hand, when aldehydes or ketones condense with hydroxy-compounds (including sugars) the products resemble sucrose in their ease of hydrolysis. Numerous isopropylidene and benzylidene derivatives may be quoted in illustration, and the reactions in which such compounds are formed have been shown to follow the course indicated below:



Applying this to the special case of carbohydrates, γ-sugars may be regarded in a new light; γ-fructose may, in fact, be nothing more than the ketonic form of the sugar and γ-glucose the corresponding aldehyde. It may well be that the aldoses and ketoses functioning in their primary capacity as aldehyde and ketone are the reactive sugars in Nature, but this much seems clear. No naturally-occurring compound of glucose, whether glucoside, disaccharide or polysaccharide, has been found to contain the γ-glucose structure and the reactive type of the sugar has hitherto been obtained only under artificial conditions. In sharp contrast, the two most important nature derivatives of fructose are each based on γ-fructose which may possibly prove to be the connecting link in natural processes whereby the interconversion of ketoses and aldoses is effected.

The structural studies which I have laid before you are admittedly incomplete and the various formulae now suggested represent only stages in knowledge and thus have no claim to finality. Much reliance has been placed on the validity of the methylation process as a means of determining constitution, but it has to be remembered that in the carbohydrates most speculation of the kind is now based on results obtained by this particular method. If I may add one personal note, it is that I have submitted these results not as an individual contribution but as the work of a loyal research school. The colleagues, collaborators and students who have played their part in the laborious work involved, if unnamed now, are always gratefully remembered by me; but memory dwells specially on the one who carried the spirit of chemical research to St. Andrews and who provided the facilities and the enthusiasm which it has been my good fortune to share. I refer to Thomas Purdie.