CCCXLVIII.—*The Structure of Carbohydrates and their Optical Rotatory Power. Part I. General Introduction.**

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(1) *The Supposed Validity of the "Principle" of Optical Superposition.*—C. S. Hudson has investigated the rotational data of the sugars and has found that, for a limited range of nearly related substances, the magnitudes are more or less additive. So long as these comparisons are confined to compounds which are parallel in properties and constitution, as determined by direct chemical methods, no sharp differences of opinion may arise from the inferences to be drawn from this kind of inquiry. It should be recognised, however, that there is no experimental sanction for the view that optical rotation is uniformly an additive property. The earlier attempts of Guye and of Walden to establish the "principle" of optical superposition ended in failure when applied to a wider range of compounds, and even the data by which these authors supported the suggestion of van 't Hoff were adversely criticised by later workers. Rosanoff (*J. Amer. Chem. Soc.*, 1906, **28**, 525; 1907, **29**, 536), whose services to sugar chemistry are widely known, has declared that substances of like structure but different stereochemical form need show no essential relationship between their optical rotatory powers. He summarised these views in the dictum, repeated with the following emphasis in each of his papers: "The optical rotatory power of an asymmetric carbon atom depends upon the composition, constitution, and *configuration* of each of its four groups." Dissimilarity in configuration is therefore recognised by

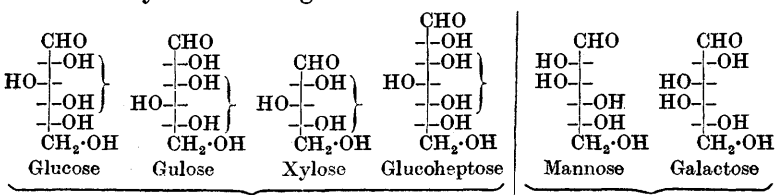
* A *résumé* of this communication appeared in *Nature*, Aug. 16th, 1930, p. 238.

Rosanoff as an impediment to the completely additive character of the physical values of optical rotatory power.

Since optical rotations of sugars have usually been recorded for one solvent only and for light of one selected wave-length, the need for caution in interpreting these data requires no further emphasis. Supposed discrepancies in the rotational constants of tetramethyl α-methylmannoside disappear entirely when alcohol as solvent is substituted for water (see section 6), and in several cases of methylated lactones profound differences of rotation appear when the solvent is varied (section 4). The effect of temperature on the rotations of many sugars is very marked. Like conditions of states such as temperature and concentration may provide one standard for comparison of rotational data, but there are no grounds for excluding alternative standards such as, for example, like conditions of viscosity or refractive index.

(2) *Hudson's Classification of the Sugars into Structural Categories.* —C. S. Hudson (*J. Amer. Chem. Soc.*, 1930, **52**, 1680, 1707) has not claimed for the " principle " of optical superposition any application outside the sugar group, but he has sought the evidence for a general and close approximation to it within this group. His procedure is to assume that it is approximately true and to explain any departure from it by difference in structure. He finds there are two (or three) categories of sugars, (a) those in which the additive principle seems to hold on the supposition of one general structure, and (b) those in which different additive relationships seem to hold. The latter, on his view, are given a different structure. By assuming that these two categories are separate and distinct, he contends he is able to use the rotation data for the calculation of rotations of other sugars in either category. Should the latter predictions be realised by the isolation of new compounds having approximately the calculated rotations, then he suggests that the validity of the additive principle is proved and that the conception of two categories of structure for the sugars is also proved.

This division of the six common aldoses into categories may be illustrated by the following :



Hudson has reported his failure to classify all the normal forms of

these six sugars into one category on the basis of rotational magnitudes and has assigned one common structure to the first four (category B), and a different structure to galactose and to one of the normal varieties of mannose (category A). His use of the term " structure " is the generally recognised one and is not to be confused in meaning with configuration. A comparison of the formulæ in category B, however, reveals some kind of restricted similarity in the configurations at three of the carbon centres (shown by a bracket). This order of grouping does not appear in the two sugars in category A. But if one writes any common ring structure, say the 1 : 5, for the α- and β-forms of all six sugars, other similarities appear, and the categories to some extent overlap. For example, some arrangements of groups in β-mannose resemble those in α-gulose and in α(α-)glucoheptose, whilst α-galactose resembles α-mannose. Also arabinose may be brought into the comparison, although for the moment Hudson has suspended judgment on the structure of this sugar.

According to Rosanoff's dictum (section 1) the above failure to classify the six sugars in one category on the basis of rotational magnitudes is only to be expected, since Rosanoff has recognised that, with substances possessing a common structure, the disparity in their configurational relationships alone provides a sufficient explanation of their departure from a common additive principle. The question at once arises, what experimental justification has Hudson for suggesting that structural and not configurational differences are responsible for the lack of uniformity in the possible application of the principle of optical superposition to all the six sugars? We confess that we have found no such sanction for this *ad hoc* suggestion in any of Hudson's writings. It seems to us to be reasonable to test a hypothesis and the deductions made from it by comparing them with experimental results, but when additional and entirely unproven assumptions are superimposed on the first to account for discrepancies which arise between the deductions and the observed facts (rotational values), these additional assumptions can have no assurance of validity. This argument appears to us to go to the root of Hudson's methods of reasoning. He has found that rotational magnitudes furnish two (or three) categories for the sugar group, but we are unable to discover any justification for the interpretation that these differences are connected with a difference in chemical structure of the substances.

(3) *The Ring Systems selected by Hudson.*—The further hypothesis advanced by Hudson is that, not only are the rotational differences discernible in sugars of categories A and B to be correlated with structural differences, but this structural difference has

reference to the number of atoms constituting the sugar ring. Again we can find in his argument no readily deducible sanction for this view beyond the statement that two categories of rotational data connote sugars of a different order. In the preceding section (2) it is seen that on Rosanoff's view differing systems of configuration would account entirely for these distinctions, but it may be added that where aggregates of *cis*-hydroxyl groups occur in spatial proximity a mutual interaction not unrelated to co-ordination is possible and it is also conceivable that the atoms of the sugar ring may be subject to slight strain (compare Haworth and Hirst, J., 1928, 1221). We have interpreted the exceptional properties of the third varieties of acetylated α-methylrhamnoside and α-methylmannoside in this way, since by methylation methods it is found that three forms of these substances having identical ring structures exist instead of the expected two forms, and the properties of each enabled us to show that groups at carbons 1 and 2 interact (Bott, Haworth, and Hirst, this vol., p. 1395; Freudenberg and Scholz, *Ber.*, 1930, **63**, 1969; Braun, *ibid.*, p. 1972). Still another hypothesis of Hudson is that the sugars of category B possess the 1 : 5- or pyranose-ring and those of category A the 1 : 4- or furanose-ring, with, however, the distinction that β-mannose is placed in category B and α-mannose in category A. These two interconvertible, mutarotating forms of mannose are thus assigned different ring structures. This hypothesis is, indeed, the primary basis of Hudson's more recent calculations of additive values (see section 6).

(4) *Variations of the Initial Rotations of Lactones in Different Solvents.*—The sugars as a group suffer from the disability that they are insoluble in most organic media and their rotations are only fully recorded for water as solvent. The effect on the rotation of varying the solvent cannot therefore be adequately studied for the unsubstituted sugars. Again, the fully substituted acetates are exceedingly sparingly soluble in water and their rotations are usually measured in chloroform. Hudson has utilised the values taken in a single solvent in each case for his computations. Methylated sugars and their derivatives, on the other hand, dissolve freely in most organic solvents and they also remain easily soluble in water, so that the effect of different solvents can best be observed with these derivatives. We have been engaged during the past four years upon an intensive study of the rotatory dispersion of all varieties of sugars and their compounds in many solvents and have encountered many significant results which will be published when the series is complete.

During this investigation the interesting fact has emerged (Part V;

this vol., p. 2659) that, whereas the methylated lactones of the glucose and xylose group change their rotations to a moderate extent in different solvents, and those of the galactose and arabinose series to a greater extent, yet in the mannose series, comprising also lyxose and rhamnose, the variations are astonishingly great, amounting to as much as 150° with complete reversal of sign. In the cases cited, the rotation in water is the initial rotation for 100% lactone, but this value is, of course, changed as hydrolysis proceeds to the corresponding acid. In the cases cited for the other solvents, the initial rotation remains constant, since owing to the absence of water no hydrolysis supervenes and the lactones are quantitatively recoverable on evaporation of the solvent. The values for all four solvents are therefore comparable.

Specific Rotations $[\alpha]_D$ *of Completely Methylated Forms of the Following Lactones in Different Solutions.*

	Lactones.	Solvent: Water.	Chloroform.	Ether.	Benzene.
Mannose series.	δ-Mannono (*d*-)	+150°	+ 59·5°	+ 35°	+ 20°
	γ-Mannono (*d*-)	+ 65	− 10	− 36	− 50
	δ-Lyxono (*d*-)	+ 35·5	− 60	− 87	−102
	γ-Lyxono (*d*-)	+ 82·5	− 28	− 70	− 70
	δ-Rhamnono (*l*-)	−130	− 68	− 39	− 15
	γ-Rhamnono (*l*-)	− 56·5	+ 13	+ 65	+ 87
Glucose series.	δ-Glucono (*d*-)	+ 98	+103	+123	+121
	γ-Glucono (*d*-)	+ 62	+ 42	+ 67	+ 68
	δ-Xylono (*d*-)	0	+ 9	+ 12	+ 17
	γ-Xylono (*d*-)	+ 88	+ 81	+ 84	+106
Galactose series.	δ-Galactono (*d*-)	+153	+101	+ 96	+128
	γ-Galactono (*d*-)	− 34	− 13	− 11	− 11
	δ-Arabono (*l*-)	+181	+125	+105	+166
	γ-Arabono (*l*-)	− 44	− 9	− 3	+ 16

The effect of solvent upon the rotational data of these sugar derivatives is so marked as to render perilous the attempt to decipher from them any additive rule or principle. To put the facts another way, the latitude offered by these figures may provide a basis for a wide selection of rules. These effects are attributable to the underlying differences of configuration. They fall definitely into three classes: (1) the glucose series, within which variations are minimised; (2) the galactose series, in which they are much more definitely marked; (3) the mannose series, in which the effects are enormous and incomprehensible. It is significant that it is in the last series that Hudson finds the widest discrepancies in the rotations of the simple sugars and the glycosides, and his interpretations demand alterations in the ring systems of galactose, mannose, lyxose and rhamnose. Yet in the above series of lactones there can be no suggestion of interchange of ring structure from solvent to solvent,

since there are no free hydroxyl positions available for such an interchange. We defer for future discussion the many interesting inferences which may be drawn from these data, but the point now to be emphasised is, to take one case only, that where rotational values so remote from one another as + 65° and − 50° are given by one simple derivative of mannose any attempt to establish a fixed arithmetical or additive relation for the individual asymmetric centres of mannose derivatives will have a doubtful validity. For this reason we suggest that no special significance attaches to the rotation value of Dale's second calcium chloride compound of mannose, and we are not impressed by interpretations which emphasise its importance. A point of some importance is that even Hudson's " lactone rule " ceases to be generally applicable to a wide range of substances and of solvents. Although it appears to hold when the solvent is water, it breaks down for the alternative solvent selected as standard by Hudson, namely, chloroform.

No validity necessarily attaches to any *ad hoc* hypothesis which is intended to explain discrepancies in the additive relationships applied to the free sugars or their glycosides. Arguments from such statistical data may, as in other fields of statistical inquiry, lead to invalid conclusions unless the causes underlying such data are known with certainty.

(5) *Nature of the Structural Proofs accepted by Hudson.*—We have examined the reasons given by Hudson for preferring the 1 : 5-ring for normal sugars of the glucose class (3) and although his present view of the structure of glucose now coincides with that which we established five years ago (Haworth, *Nature*, 1925, **116**, 430) we are unable to find in this reconciliation any experimental confirmation of our constitutional formula. Abandoning as invalid any proofs by methylation methods, Hudson has resorted to the experimental data given by Zemplén on the constitutions of cellobiose, maltose, and lactose. It is suggested that groups migrate during methylation but not during acetylation. There is abundant evidence elsewhere, however, that acetyl groups are very prone to migrate, whereas methyl groups do not display this instability (Ohle, *Ber.*, 1924, **57**, 403; Josephson, *Annalen*, 1929, **472**, 217; Haworth, Hirst, and Teece, this vol., p. 1405). Hudson has accepted Zemplén's observation that the point of junction of the two hexose units in cellobiose, maltose, and lactose is at position 4 in the reducing hexose unit (*Ber.*, 1926, **59**, 1254; 1927, **60**, 1309, 1555). We agree this is so because of our independent methylation data (J., 1926, 3094; 1927, 544, 2809). Taken alone, the method of Zemplén's proof is, however, negative in character, since it depends (1) on the degradation of these three bioses to a hexose-tetrose and

(2) on the failure of this hexose-tetrose to give an osazone, the inference being that the 4-position, becoming the 2-position in a tetrose residue, does not have a hydroxyl group at this position to comply with osazone formation. But, as we have previously stated (J., 1927, 545), there may be many tangible reasons other than the one selected to account for a negative result such as the non-formation of an osazone. Indeed, this dubiety was illustrated when Zemplén applied the same method to melibiose. Here the derived hexose-pentose also failed to yield an osazone, but the difficulty was one of experiment and isolation and not of structure; the 3-position was allotted by Zemplén to the biose junction in melibiose, although Haworth, Loach, and Long (J., 1927, 3146) and Helferich (*Annalen*, 1928, **465**, 170) proved the junction to be in position 6. The latter result and not that of Zemplén is accepted and utilised by Hudson in his formula for melibiose. One cannot, however, discard the type of negative proof furnished by Zemplén in one case without impairing the validity of its application to other cases. It follows logically, then, that apart from the proof by methylation data which we have provided for the 4-position of the biose link in cellobiose, maltose, and lactose, there is no valid chemical experiment which defines the position of attachment of the biose link at any one position.

Even if it be accepted that Zemplén's observations on the 4-position of the linking of the two hexoses in the cases of cellobiose, maltose, and lactose are valid, they provide no evidence of ring structure whatever, since the ring junctions may occupy any positions in the reducing hexose unit except that of 1 and 4. By what choice is the 1 : 5-ring selected rather than 1 : 6 or 1 : 3 or even 1 : 2 (if osazone formation occurs through the aldehyde phase)? There exist no data for any choice other than that provided from methylation methods, lactone formation and degradation, which we have supplied. It emerges, therefore, that Hudson can provide no evidence for the 1 : 5-ring in any of the sugars to which he assigns it except from the methylation data which he discards. The terms he uses for ring structures are deprived of any background of proof. The only other chemical experiments quoted by Hudson as a foundation for the 1 : 5-ring in normal glucose or its derivatives is the isolation (Purves, *J. Amer. Chem. Soc.*, 1929, **59**, 3619, 3631) of a normal β-thiophenylglucoside from β-thiophenylcellobioside, -maltoside, and -lactoside by acid hydrolysis, and also the isolation (Fischer and Armstrong, *Ber.*, 1901, **34**, 2885) of β-methylglucoside from β-methylmaltoside by enzyme cleavage. These data are considered also in section 12. They furnish, however, no evidence that β-thiophenylglucoside or β-methylglucoside

possesses a 1 : 5-ring structure rather than a 1 : 2, 1 : 3, or 1 : 6 structure. Since, also, the Zemplén formula for melibiose is rejected by Hudson and since the validity of his similar experimental proofs for the 4-position of the biose link in the remaining disaccharides is thereby invaded, there appears no reason for excluding the 1 : 4-ring structure for β-methylglucoside and β-thiophenylglucoside. It appears, therefore, that so long as Hudson disallows the proofs by methylation data he is confronted with the dilemma that the 1 : 2-, 1 : 3-, 1 : 4-, 1 : 5-, or 1 : 6-ring structure may apply to the latter glucosides to which he has arbitrarily allotted a 1 : 5-ring. It follows logically that no classification of ring structure in any definable category can be offered for any of the hexoses, and structural questions fall once more into the limbo of speculation from which we aver that the intimate and direct chemical methods which we have applied had rescued them. Apart from the latter experiments there exists no experimental basis for a constitutional proof possessing standards similar to those which are accepted for all other groups of organic substances.

(6) *The Rotations of α- and β-Mannose.*—A fundamental thesis in Hudson's recent scheme is the denial of structural identity to α- and β-mannose and to all their derivatives. The reason offered for this view is that the specific rotations (in water) of α- and β-mannose are respectively + 30° and − 17°, whereas for α- and β-glucose the corresponding values are + 113° and + 19° : the latter differ by 94°, whilst the difference for the two mannoses is only 47°. Several other sugars show a difference corresponding to that of mannose, and these distinctions extend also to the α- and β-methylmannosides, their tetra-acetates, and, as we have now shown in a following paper (Part IV; Bott, Haworth, and Hirst), to the tetramethyl derivatives. Their rotational data being expressed as molecular values (specific rotation × mol. wt.), the two mannoses show a molecular difference of 47 × 180 = 8460, as compared with a corresponding difference of about 17,000 for the two glucoses. These molecular rotational differences are expressed by Hudson as $2a_{OH}$ values and the deficiency (17,000—8460) shown by comparing glucoses with mannoses is of the order of 8500. The same order of deficiency (9000) is found on comparing the α- and β-methylglucosides ($2a_{OMe}$ values) with the α- and β-methylmannosides. We have supplemented this comparison by taking the cases of the tetramethyl derivatives of α- and β-methylmannosides, which show a discrepancy of a similar order of magnitude (11,500).

Because of these deficiencies in molecular rotation values of α- and β-mannose as compared with glucose and some other sugars, Hudson makes the hypothesis that α- and β-mannose cannot be

merely stereoisomerides of one common structural form. Selecting the α-forms as being anomalous, he ascribes to α-mannose and α-methylmannoside a different ring form from β-mannose and α- and β-glucose and their glucosides, and also their tetra-acetates. He assumes now that the true structural isomeride of β-mannose (and α- and β-glucose, etc.) should be an unknown form of α-mannose having $[\alpha]_D + 77°$, since such a value would bring the molecular rotational difference between this α- and the known β-form into line with those that are found for the two glucoses. Corresponding to the unknown α-mannose he considers there should also be a new variety of α-methylmannoside having $[\alpha]_D + 125°$, and special attention is focused on this hypothesis because of the implications discussed in section 12. This initial hypothesis enables him to straighten out many of the anomalous rotations displayed by many of the sugars and it leads him to adopt the classification which extends to other known sugars.

Opposed to this view is the direct chemical evidence on the structure of α-methylmannoside. We have found (Goodyear and Haworth, J., 1927, 3136; Bott, Haworth, and Hirst, this vol., p. 2653) that both α- and β-methylmannosides yield crystalline tetramethyl derivatives which on hydrolysis give one and the same tetramethyl mannose. This is epimerised to tetramethyl glucose by alkali by the Lobry de Bruyn and van Ekenstein reaction (Wolfrom and Lewis, *J. Amer. Chem. Soc.*, 1928, **50**, 837), and also gives rise on oxidation to a crystalline tetramethyl *d*-mannonolactone. The latter is degraded by oxidation to *d*-trimethoxyglutaric acid, so its ring structure can only be that of a 1 : 5- or pyranose-form. Hudson agrees that the part of this evidence which is concerned with the structure of tetramethyl mannose is conclusive, but he claims other parts of the evidence as a decisive proof that change of ring has occurred during the methylation of α-methylmannoside. It is therefore suggested that dilute alkali and methyl sulphate (or Purdie's reagents) do not change the ring in β-methylmannoside but that they do in α-methylmannoside, and that the latter then assumes the same ring form as β-methylmannoside. The tetramethyl derivatives of these mannosides, which are crystalline, should then on Hudson's admission have the same ring forms, since they yield the same crystalline tetramethyl sugar on hydrolysis. This only leads to a dilemma, since we have found that the tetramethyl α- and β-methylmannosides show about the same order of molecular rotational deficiency (11,500) as do the normal forms of α- and β-mannose (8500) or α- and β-methylmannosides (9000) and yet it is the latter anomalies which led Hudson to allocate different ring forms to the mannosides. It may further be added that all

the figures so far discussed represent the rotations taken in water at 20°. When we determined the rotations of tetramethyl α- and β-methylmannosides in ethyl alcohol, we found that the discrepancy of the molecular rotational differences disappeared almost entirely, and these two substances fell into line with the water or alcohol values for several other methylated glycosides, as will be seen from the accompanying table.

TABLE I.

Molecular Rotational Differences ($2a_{OMe}$ *Values*) *for Completely Methylated* α- *and* β-*Derivatives of the Normal Sugars.*

Sugar.	In water.	In other solvents.
Glucose	41,100	42,800 (EtOH)
Galactose	42,000	42,500 ,,
Arabinose	45,000	41,200 (MeOH)
Xylose	40,170	39,300 ($CHCl_3$)
Mannose	30,600	39,500 (EtOH)
		36,000 ($CHCl_3$)

Hudson must either explain the persistence of this deficiency of 11,500 for tetramethyl α- and β-methylmannosides by the same hypothesis of difference in ring structure which he suggests for α- and β-mannose, or admit that the deficiency is of no consequence and cannot be correlated with structural form.

(7) *The Isolation of a New Variety of α-Methylmannoside and its Comparison with Normal Forms.*—Last year we isolated two new crystalline varieties of ethylglucoside, which were designated as furanosides or 1 : 4-ring types. These differed from the previously known normal or pyranoside forms both in rotation and in the ease with which they underwent hydrolysis to glucose. They were hydrolysed a hundred times as rapidly as the pyranosides under identical conditions (Haworth and Porter, J., 1929, 2796). Recently we have also isolated a new crystalline variety of α-methylmannoside, and both its mode of formation from mannose dicarbonate and its capacity to combine with acetone directly in the presence of anhydrous copper sulphate indicated the presence of two pairs of *cis*-hydroxyl groups, which are best accommodated by adopting the 1 : 4- or furanoside ring. The presence of this ring form was confirmed by methylation and oxidation methods, and we designated the compound α-methylmannofuranoside, $[\alpha]_D$ + 113°. The substance is hydrolysed about 100 times as rapidly as the normal α-methylmannoside, $[\alpha]_D$ + 79°, and this clear distinction between the rates of hydrolysis of the two forms is preserved in their tetramethyl derivatives. If, as Hudson avers, a change of ring structure occurs when the normal form, $[\alpha]_D$ + 79°, passes into its tetramethyl derivative, there is no corresponding change of chemical or physical

property to proclaim it, since the rate of hydrolysis is of the same order. We have already shown also that the discrepancy in the molecular rotational difference between its α- and β-forms, whether methylated or unmethylated, remains of the same order.

Again, the new α-methylmannoside, $[\alpha]_D$ + 113°, gives a quantitative yield of its crystalline tetramethyl derivative, and this preserves the characteristic property of its unmethylated form in being readily hydrolysed with N/100-acid. On hydrolysis it yields a tetramethyl mannose, $[\alpha]_D$ + 43° (equil.), which gives on oxidation the same crystalline tetramethyl γ-mannonolactone (five-atom ring form) as that obtained from the interconversion of mannose-diacetone (Haworth, Hirst, and Webb, this vol., p. 651).

Both the normal and the new variety of α-methylmannoside are unaffected by hot 15% alkali during two hours. There seems, therefore, no reason to suspect instability of the ring system of either form by methylation with very dilute alkali and methyl sulphate or with Purdie's reagents.

We have measured the comparative rates of hydrolysis of pure specimens of glycosides and their methylated derivatives of the two general types of ring structure already established by methods which we have published. The following figures (in terms of minutes and decimal logarithms) represent the approximate order of the reaction velocities determined under similar conditions of heating at 95—100° with N/100-hydrochloric acid.

Pyranosides (1 : 5-ring).	$k \times 10^5$.	Furanosides (1 : 4-ring).	$k \times 10^5$.
α-Methylglucoside	25	Sucrose	5000
Tetramethyl α-methyl-glucoside	4	Octamethyl sucrose	1000
β-Methylglucoside	30	β-Ethylglucoside	5300
Tetramethyl β-methyl-glucoside	10	Tetramethyl β-ethyl-glucoside	1400
α-Methylmannoside	10	α-Methylmannoside	1500
Tetramethyl α-methyl-mannoside	4	Tetramethyl α-methyl-mannoside	250
α-Methylgalactoside	23		
Tetramethyl α-methyl-galactoside	4		

We derive from these figures the simple rule that, under these conditions and for the range of substances selected by Hudson for his attack on our structural formulæ, the glycosides preserve, in general, the same order of stability in their methylated forms, and that, for glycosides of the same sugar, the furanoside form is much more easily hydrolysed than the corresponding pyranoside form. Such comparisons have only been rendered possible by the recent isolation of the pure homogeneous furanosides of the glucose and mannose series. The earlier derivatives of γ-sugars described in

the literature were not homogeneous but contained varying proportions of the pyranose isomerides which prevent their being used in the above comparisons.

It may be deduced from the above figures that the reaction velocity, 10, for α-methylmannoside precludes its classification among the furanosides and that the facts are against its classification by Hudson in a category different from that of α-methylglucoside. Again, it is seen that the tetramethyl derivative having a reaction velocity of 4 cannot possibly belong to a different classification from that of the parent form of α-methylmannoside. Hudson similarly ascribes to α-methylgalactoside a furanoside structure which would place it in the wrong category in the above table. It is also evident that sucrose, which contains a fructofuranoside residue, has a value similar to that determined for the crystalline β-ethylglucofuranoside. Hudson has doubted whether this would be found to be the case, and has placed sucrose in a separate structural category with a 1 : 3-ring for the fructose residue. His analogy between sucrose and the third variety of tetra-acetyl β-methylmannoside does not hold inasmuch as the latter is hydrolysed instantaneously at 20° with acid so dilute as $N/1000$. We have shown (section 3) that this is a derivative of orthocarbonic ester and retains also the pyranose ring (Bott, Haworth, and Hirst, *loc. cit.*).

(8) *Glucose and Mannose Derivatives and "Epimeric Differences."*—When we published our work on the isolation of the two ethylglucofuranosides (1 : 4) we used the fact of their isolation as an argument against Hudson's opinion that the ordinary glucose possessed a 1 : 4-ring and the known normal methylglucosides had the constitution of furanosides. There is no need to continue this argument further, inasmuch as Hudson, in the two published papers now under discussion, has become reconciled to our original view. It is noteworthy, however, that he has claimed this isolation of the above new forms as "a striking experimental confirmation of his prediction" and suggests that "they prove at the same time, through the epimeric difference, that α-methyl *d*-mannoside (+ 79°) possesses the 1 : 4-ring." It is conceivable that Hudson may also claim our isolation of the new form of α-methylmannoside (1 : 4) (+ 113°) as a confirmation of his prediction of the hypothetical form (1 : 5) (+ 125°), since the margin of error is similar to that (+ 12°) which we observed for the glucofuranosides. We believe Hudson will perceive the danger of this course in that the "constancy" of his value for "epimeric differences" becomes at once invalid. Assuming that this course is taken, it is seen below that it leads to no constant difference at all.

Stated as originally predicted (with Hudson's later modification of ring), these values of $[\alpha]_D$ were :

α-Methylglucoside (1 : 5)	+159°	α-Methylglucoside (1 : 4) (unknown)	+115°
α-Methylmannoside (1 : 5) (unknown)	+125	α-Methylmannoside (1 : 4)	+ 79
Epimeric diff.	34		36

Assuming now that our new isolated forms fulfil these predictions, and inserting the observed values for the " unknown forms," we have :

α-Methylglucoside (1 : 5)	+159°	α-Methylglucoside (1 : 4) (calc. from the ethylglucoside)	+101°
α-Methylmannoside (1 : 5)	+113	α-Methylmannoside (1 : 4)	+ 79
Epimeric diff.	46		22

The two margins of error of 12° and 13° between observed and calculated values are seen to operate in a contrary sense and to endanger the use of " epimeric difference " as a valid factor in the classification of the numerous sugars to which Hudson has applied it. When the true ring structures as we have determined them are compared as follows, constancy of epimeric difference ceases to have any rational meaning :

	$[\alpha]_D$.		$[\alpha]_D$.
α-Methylglucoside (1 : 5)	+159°	α-Methylglucoside (1 : 4)	+101°
α-Methylmannoside (1 : 5)	+ 79	α-Methylmannoside (1 : 4)	+113
Epimeric diff.	80		− 12

(9) *Hudson's New Formulæ for Disaccharides and Polysaccharides.* —By computations from rotational magnitudes Hudson has reached the conclusion that some constitutional formulæ we have determined should be modified. His choice of standards appears to us to be so elastic that we have been led to make other calculations which, if one were convinced of the validity of his method, would lead to different views of structure from those he has adopted.

The figures for the computation of ring structures in disaccharides given in the accompanying table have been calculated upon the following assumptions : (a) that the rotational value of the lactonyl grouping in the biose linking is the same as that in α-methyl-glucoside (18,500) ; (b) that when the biose linking involves the 6th carbon of a sugar the rotational effect of that sugar residue is the same as it is in the free sugar ; (c) that the ring structures in the component hexoses are all 1 : 5, as we have shown to be the case by methylation methods. Cellobiose is taken as standard [4-β-glucosido-(1 : 5)-β-glucose (1 : 5) having $[\alpha]_D$ + 16°] and from it the value for the substituted glucose residue present in cellobiose is calculated by means of (a). In certain cases (marked *) epimeric differences have been used and where this has been done the observed value 15,400 has been employed for α-glucose (1 : 5) *minus*

α-mannose (1 : 5) differences and the observed value 6,400 for β-glucose (1 : 5) *minus* β-mannose (1 : 5) differences.

Sugar.	$[\alpha]_D$ obs.	$[\alpha]_D$ calc.	Hudson's values.
α-Maltose	118°	124°	119°
β-Gentiobiose	−11	−9	Taken as standard
β-Lactose	35	34	—
α-Lactose	90	86	—
β-Melibiose	124	117	137
*4-β-Glucosido-α-mannose	20·5	27* 22 †	52
*4-β-Glucosido-β-mannose	0	−3*	−2
*4-β-Galactosido-α-mannose	38	45*	70
*4-β-Galactosido-β-mannose	16	16*	15
Trehalose	197	179	Taken as standard
β-Cellobiose	16	—	—

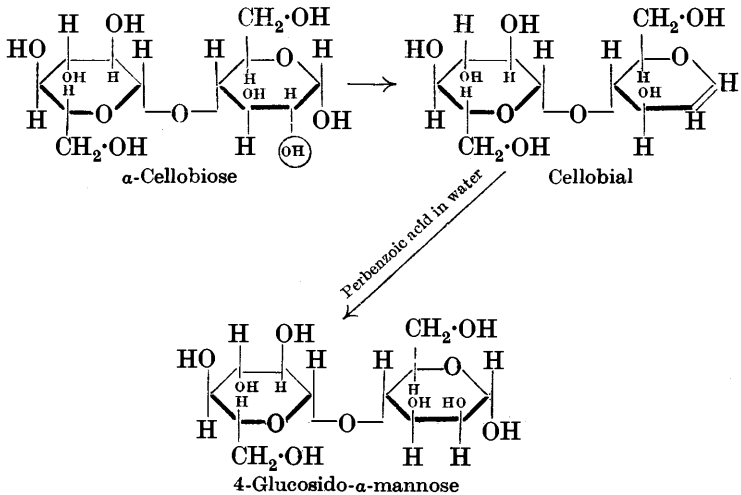
† Calculated from 4-β-galactosido-α-mannose by application of (Ga–G), difference between the values for galactose and glucose.

The agreement between the observed and the calculated values here is closer and more comprehensive than is the case when Hudson's method of calculation is used, which involves changing the ring form in maltose, lactose, and other bioses. The greatest divergence, 18° for trehalose, is very little more than Hudson's divergence of 13° for melibiose. It is seen, then, that better agreement between calculated and observed values can be attained than by altering ring-forms of the hexose components. No particular importance as regards structural questions is, in our opinion, to be attached to these agreements, but they are recorded in order to demonstrate that a scheme of approximately accurate rotation values can be calculated upon assumptions which are directly opposed in essential features to the structural views of Hudson. The elasticity of the methods of " proving " the ring structure of carbohydrates by rotational data is obvious.

At this point it is convenient to refer to Hudson's criticism of Charlton, Haworth, and Hickinbottom (J., 1927, 1530) for their use of the lactonyl rotation value (18,500) in obtaining supplementary evidence for the presence of an α- or a β-linking in a melibiose. In that paper we made no claim whatever that the ring structure of melibiose could be determined arithmetically or even supported by such a method. Our tentative use of rotation data was purely qualitative and involved only rotation differences which could reasonably be supposed to be either very large or very small to differentiate an α- from a β-form. It is immaterial whether the above lactonyl constant or the new one now offered by Hudson be used for this purpose, since the conclusion we then reached would be the same. The lactonyl constant we used, however, is that which has given rise to the above table, which, for us, possesses no structural meaning.

(10) *A "Crucial Test" applied by Hudson in Support of his Structural Scheme.*—The stimulation given by Hudson's theoretical views to the preparation of sugars and their derivatives in a condition of greater purity has led to many notable advances in technique, and whilst we are unable to share his interpretations we welcome the incitement they have given to the isolation of new compounds. Among these are the acetohalogeno-derivatives of 4-glucosidomannose (Brauns, *J. Amer. Chem. Soc.*, 1926, **48**, 2776). This biose was first prepared by Bergmann and Schotte (*Ber.*,. 1921, **54**, 1564) by the inversion of the 2-hydroxyl group in the reducing unit in cellobiose. This is accomplished by treating acetobromocellobiose with zinc dust; the unsaturated product, cellobial, undergoes hydroxylation with aqueous perbenzoic acid to give 4-glucosido-α-mannose. A similar transformation of lactose, through lactal, gives 4-galactosidomannose (Bergmann, *Annalen*, 1923, **434**, 79).

Now the cellobiose structure which Hudson adopts and by which he explains these transformations happens to be identical with that which we had previously established by experiment. This requires both glucose units to be pyranose forms and locates the biose junction at position 4 of the reducing unit.



It is not disputed that during these transformations the biose linking remains fixed and therefore that there can be no ring shift in the reducing residue from a 1 : 5- to a 1 : 4-position. The conversion of a glucosido-α-glucose (1 : 5) into a glucosido-α-mannose (1 : 5) is therefore a genuine configurational or epimeric change, and Hudson finds in these two compounds a means of testing, by the use of "epimeric differences" (see section 8), his hypothesis that the

1 : 5-mannose residue is not that of the ordinary form of α-mannose having $[\alpha]_D$ + 30° but is that of the hypothetical α-mannose (calculated $[\alpha]_D$ + 77°; see section 6). This conception he has endeavoured to put to the " crucial test " by comparing the rotations of the four acetohalogeno-derivatives (fluoro-, chloro-, bromo-, and iodo-) of cellobiose (4-glucosidoglucose) with those of the epimeric forms from 4-glucosidomannose. Utilising this factor of " epimeric difference" derived from the comparison of the rotation of tetra-acetyl α-methylglucoside (+ 131°) with that of his hypothetical tetra-acetyl α-methylmannoside (calculated + 102°), he finds that the constant difference of 11,300 molecular units applies also in the comparison between the acetohalogeno-derivative of cellobiose and the acetohalogeno-derivatives of 4-glucosidomannose which were isolated by Brauns. For example, the calculated rotation of aceto-bromo-4-glucosido-α-mannose on this basis is + 80° and that observed is + 78°. By this " crucial test " of rotation data Hudson is completely satisfied that the foundation for the whole of his revised structural formulæ in the sugar group is confirmed. If this basis for his contention were shown to be insecure (section 11) or if the mannose residue in 4-glucosido-α-mannose were found to be that of the known α-mannose (+ 30°) and not the hypothetical form (+ 77°) (section 12), then the primary reason for his selection of a 1 : 4-ring structure for the known α-mannose (+ 30°), and for the known β-mannose (— 17°) of a 1 : 5-ring structure, would become invalid. The direct chemical methods we have applied in the determination of the structure of normal α-methylmannoside show indeed that Hudson's hypothesis is invalid, but he abandons all proofs involving methylations under the belief that ring shifts occur during the methylation process.

(11) *Rotational Data of Derivatives of 4-Glucosidomannose and 4-Galactosidomannose.*—In view of this agreement between the calculated and the observed rotations of acetohalogeno-derivatives of 4-glucosidomannose, we have investigated other derivatives of the biose which are not confined to one type. To these inquiries we have added the examination also of the allied biose, 4-galactosido-mannose, which on Hudson's basis should also possess the unknown mannose residue (+ 77°). The experimental details of these inquiries are in Parts II and III (following papers). The following table shows that the rotations of the four acetohalogeno-derivatives are in agreement with Hudson's contention. On the other hand, he might have derived from the study of five other substances in this series an arithmetical proof of the opposite contention, namely, that the α-mannose unit (+ 30°) and not the hypothetical form (+ 77°) occurs in the biose and its derivatives. The calculations

based upon this alternative hypothesis are given in the column marked (*A*).

4-Galactosido-α-mannose and its derivatives.	[α] obs.	*A* (see text).	Calc. on Hudson's hypothesis.
Free biose, α-form	+ 38°	+45°	+ 70°
α-Methylbioside	+ 66	+71	+ 96
α-Methylbioside hepta-acetate	+ 36	+26	{ + 52 / + 46 } Calc. from alternative standards
4-Glucosido-α-mannose and its derivatives.			
Free biose, α-form	+ 20	+22	+ 52
α-Methylbioside	+ 46	+53	+ 78
α-Methylbioside hepta-acetate	+ 30	+13	+ 40
Acetofluoro-biose	+ 13·6	−14	+ 13
Acetochloro-biose	+ 51	+28	+ 54·5
Acetobromo-biose	+ 78	+55	+ 80
Acetoiodo-biose	+111·5	+87	+110·5

From our own standpoint these agreements or differences furnish no grounds for any structural view of any kind, and whilst giving the figures in column *A* for the sake of interest we merely present the dilemma that the figures agree partly with Hudson's contention and partly with the directly opposite view against which he is contending. They represent the kind of variation we should expect to find in the rotations of any structurally similar series of mannose derivatives (sections 4 and 6). Comparing the molecular rotational differences we have observed for these α- and β-forms of the two bioses, we find just about the same " deficiency " which Hudson derives for ordinary α- and β-mannose (section 6). In these two compound sugars which, by reason of the presence of the biose link at position 4, cannot suffer ring shift from 1 : 5 to 1 : 4, the discrepancy in molecular rotational difference between α- and β-forms is still evident. How, then, can the argument be maintained that this discrepancy betokens a 1 : 4-ring for α-mannose and a 1 : 5-ring for β-mannose? The " normal " molecular rotational difference for glucose and lactose is included in the table for comparison.

Molecular Rotational Differences ($2a_{OH}$ *values*) *between* α- *and* β-*Forms.*

4-Glucosido-{α/β}-mannose	6,840
4-Galactosido-{α/β}-mannose	7,500
{α/β}-Mannose	8,500
{α/β}-Lactose	18,800
{α/β}-Glucose	17,000

In addition to the above, the values of the " epimeric differences " required by Hudson's scheme are not found in the following important instances: penta-acetyl α-mannose and penta-acetyl α-glucose, octa-acetyl 4-glucosido-α-mannose and octa-acetyl α-cellobiose,

4-glucosido-α-mannose and α-cellobiose, 4-galactosido-α-mannose and α-lactose, 4-glucosido-α-methylmannoside and α-methylcellobioside, 4-galactosido-α-methylmannoside and α-methyl-lactoside (calc. value), hepta-acetyl 4-glucosido-α-methylmannoside and hepta-acetyl α-methylcellobioside, hepta-acetyl 4-galactosido-α-methylmannoside and hepta-acetyl α-methyl-lactoside (calc. value). Agreement between the observed and the calculated values is found only amongst the acetylated halogeno-derivatives of cellobiose and 4-glucosido-mannose (section 10), but even here discrepancies are shown amounting to 3000 units of molecular rotation.

(12) *The Ring Structure of the Mannose Residue in 4-Glucosido-α-methylmannoside and in 4-Galactosido-α-methylmannoside.*—Since Hudson disregards the evidence of ring structure derived from methylated compounds, we have decided to utilise here only that kind of experimental procedure which he approves and has himself employed in elaborating his structural scheme. We have done this advisedly, because there seems no reason to prolong the argument beyond the present series of papers if a genuinely crucial test which is satisfactory to Hudson can be devised. This we have naturally sought in his own papers and we have chosen the experimental method on which he rests his claim to have proved that the normal β-methylglucoside occurs as a residue in β-methylmaltoside and therefore in maltose, and that normal β-thiophenylglucoside occurs in the thiophenyl derivatives of cellobiose, lactose, and maltose. His procedure here is to accept the evidence that β-methylmaltoside yields the normal β-methylglucoside by enzyme hydrolysis, it having been ascertained that the enzyme is without effect on the β-methylglucoside itself (Fischer and Armstrong, *Ber.*, 1901, **34**, 2885). Again, he utilised the observation of Purves (*loc. cit.*) that the normal β-thiophenylglucoside, which is itself stable to mineral acid of a definite concentration, is formed by the acid hydrolysis of each of the thiophenyl derivatives of the above bioses.

As being the less drastic of the two modes of procedure, we have adopted the method of enzyme cleavage, and since both cellobiose and lactose are hydrolysed at the biose junction by emulsin, we have used the latter enzyme for the cleavage of our biose derivatives.

The problem before us may now be stated as follows :

Leaving aside all previous proofs, by methylation methods, of the ring structure of α-methylmannoside or α-mannose, we desire to reach a conclusion on the one clear issue which is fundamental to Hudson's statistical scheme : Does the ordinary normal variety of α-mannose (+ 30°) occur as a combined residue in 4-glucosido-α-mannose and in 4-galactosido-α-mannose, or is this α-mannose residue the hypothetical variety for which Hudson has calculated

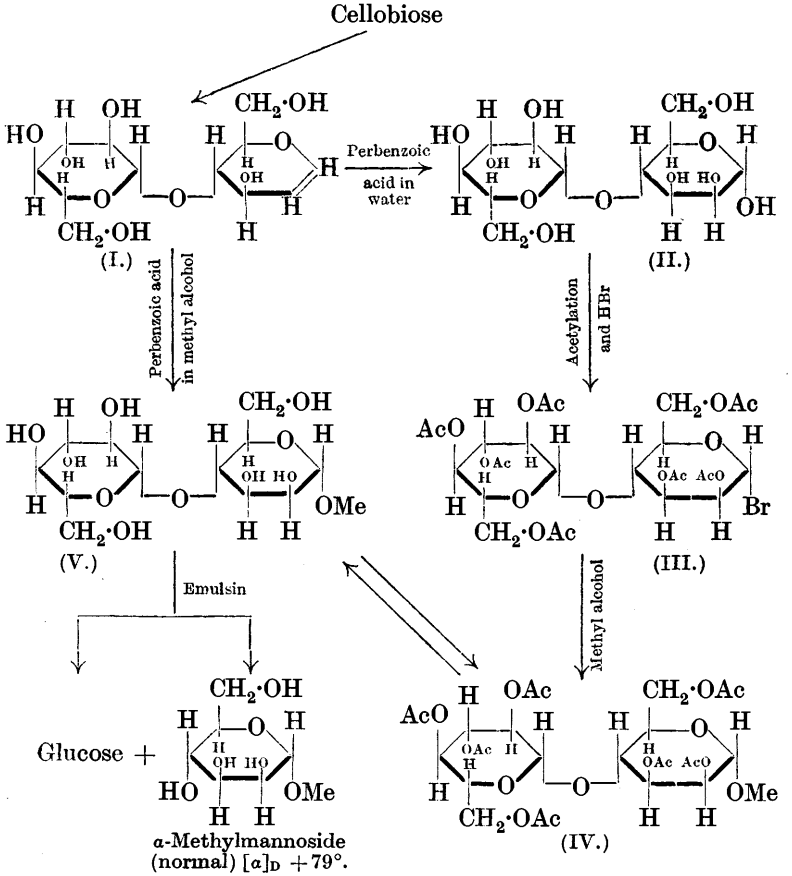
the rotation to be $[\alpha]_D$ + 77°? The fact that α-mannose (+ 30°) is obtainable by the action of emulsin on the free bioses may be considered inconclusive, since it is arguable that the other form (+ 77°) might revert to the + 30° form at the moment of its isolation. For this reason and on the strict analogy of Hudson's procedure we have operated with the methylbiosides. The known α-methylmannoside, $[\alpha]_D$ + 79°, corresponds to the known α-mannose (+ 30°), whilst Hudson has calculated for the unknown α-methylmannoside the rotation value of $[\alpha]_D$ + 125° corresponding to the unknown α-mannose (+ 77°).

The preliminary fact having been ascertained that emulsin has no action either on the ordinary normal α-methylmannoside (+ 79°) or on the new α-methylmannoside which we have recently discovered (+ 113°) (section 7), we were venturing on safe ground in applying the test in the following manner.

We prepared the α-methylglycoside of 4-glucosidomannose, namely, crystalline 4-glucosido-α-methylmannoside (V), from the pure reference compound referred to by Hudson as acetobromo-4-glucosidomannose (hepta-acetyl-4-glucosidomannosidyl bromide) (III) by condensing the latter with methyl alcohol in the presence of (a) silver carbonate and (b) quinoline. Both reagents gave the same product, namely, hepta-acetyl 4-glucosido-α-methylmannoside (IV). By utilising Hudson's reference compound as prepared by Brauns, we were certain that we were dealing with a derivative of the same disaccharide as that selected by Hudson for his " crucial test." We have also prepared the same bioside (V) by a simpler process direct from cellobial (I), utilising a method analogous to that employed by Bergmann and Schotte (*loc. cit.*) for the formation of the free biose (II) itself. These authors dihydroxylated cellobial (I) by contact with perbenzoic acid in water. We have conducted the same reaction in methyl alcohol and have obtained crystalline 4-glucosido-α-methylmannoside (V), which on acetylation passes into the hepta-acetyl derivative (IV) identical with that obtained from the acetohalogeno-derivative (III). Furthermore, the hepta-acetyl compound (IV) is transformed into (V) on deacetylation. The rotations of these products are discussed in section 11.

Taking now the 4-glucosido-α-methylmannoside (V) prepared by any of the above methods, we have sought to find whether enzyme cleavage with emulsin would yield the ordinary known variety of α-methylmannoside (+ 79°), which is the glycoside of the normal mannose (+ 30°), or whether the unknown hypothetical form of α-methylmannoside (+ 125°) could be isolated, since this is the glycoside of the unknown α-mannose calculated by Hudson to have $[\alpha]_D$ + 77°. In the latter event Hudson's contention would receive

experimental support; in the former event his theoretical basis would be rendered invalid.



We established by control experiments, using alternative methods of isolating α-methylmannoside from a mixture containing also glucose and the enzyme, that the maximum yield was about 50%. The enzyme cleavage of the 4-glucosido-α-methylmannoside proceeded under normal conditions and the essential product of the change (yield, 49% of the theoretical) was identified as the normal α-methylmannoside by its rotation, $[\alpha]_D$ + 79°, by m. p. and mixed m. p. determinations, and by conversion into the crystalline tetra-acetate, which was again identical with the acetyl derivative of the ordinary α-methylmannoside (+ 79°). The issue can no longer remain in doubt. It is established that 4-glucosido-α-methylmannoside (V) and therefore the free biose (II) itself and the aceto-halogeno-derivative from which the α-methylbioside was derived,

contain the same structural residue as that which occurs in ordinary α-mannose (+ 30°) and α-methylmannoside (+ 79°) and the theoretical basis of Hudson's calculations is deprived of experimental support in the very series which he has chosen for his crucial experimental verification. The objection that a ring shift occurs at the moment of the severance of the bioside linking by emulsin may be answered by the fact that the rotational data for these biose derivatives recorded in section 11 equally fail to support Hudson's views. Moreover, if the question of a ring shift is again raised, it is clear that the same dubiety arises from the enzyme cleavage of β-methylmaltoside and the acid cleavage of thiophenyl cellobioside, lactoside and maltoside, upon which reactions Hudson has founded his claim to have established the ring structure of glucose. And if the latter be rendered insecure, then there remain no criteria from which to derive the "epimeric differences" between normal α-methylglucoside and the hypothetical mannoside (see section 10).

The above experimental inquiry has also been extended to include the analogous case of 4-galactosido-α-mannose and its glycoside, 4-galactosido-α-methylmannoside, for which the rotational data are recorded in section 11. The latter substance suffers enzyme cleavage by emulsin at the biose junction and gives rise to galactose and α-methylmannoside (+ 79°) identical with the product isolated from 4-glucosido-α-methylmannoside. The galactoside analogue was prepared for the first time for the purpose of this investigation and was obtained by acting on lactal with perbenzoic acid in methyl alcohol, a procedure resembling that originally adopted by Bergmann for the synthesis of the free biose. The only interpretation to be applied in this case also is that neither from the rotational data of these compounds nor from the behaviour of the glycoside on hydrolysis can support be found for the hypothesis that an unknown form of α-mannoside (+ 125°) occurs in the bioside, or that a corresponding form of α-mannose (+ 77°) is present as a residue in the free biose.

The conclusion we have reached from our reinvestigation of the problem propounded by Hudson is that the interpretation of the experimental data on which we based the pyranose (1 : 5) structure of α-methylmannoside remains sound and receives further confirmation from these facts. The experiments now communicated are in complete agreement with the sugar formulæ we have established over many years by methylation studies, by lactone formation and degradation, by a comparison of the reaction velocities of glucosides under hydrolysis, and by other direct chemical methods.

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