[CONTRIBUTION FROM THE HYGIENIC LABORATORY, U. S. PUBLIC HEALTH SERVICE]

RELATIONS BETWEEN ROTATORY POWER AND STRUCTURE IN THE SUGAR GROUP. XXVI. THE RING STRUCTURES OF VARIOUS COMPOUND SUGARS[1]

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1. Introduction.—During the more recent development of our knowledge of the structures of compound natural sugars, which has come principally from the researches of Haworth, Irvine (concerning maltose and sucrose), Zemplén (concerning cellobiose, lactose, maltose, melezitose and turanose), Helferich (concerning gentiobiose) and Levene (concerning lactose and cellobiose) the writer has sought to use the results as the basis for comparing the rotations of such sugars and their derivatives in order to determine to what extent the rules of isorotation hold among them. In some comparisons the rules held closely but in the case of maltose wide divergence was apparent, a divergence so great as to suggest that the accepted structure of this sugar might be in error. In the case of sucrose, raffinose and gentianose the writer could not reconcile the ease with which the fructose molecule is split off through acid hydrolysis with the 2,5-ring structure that has been assigned to this component by Haworth from methylation studies; a 2,4-ring structure seemed far more probable from the previous assignment[2] of this ring to the easily hydrolyzed glycosides in the mannose and rhamnose series. The writer saw that the isorotation rules were not necessarily in conflict with the specific structural allocations that had come from the researches of Zemplén regarding cellobiose, lactose and maltose, of Helferich regarding gentiobiose, and of Levene regarding cellobiose and lactose, nor with that phase of the methylation researches of Haworth through which he had assigned the 4-glycosidic union to cellobiose, lactose and maltose and the 6-union to melibiose and gentiobiose. On the other hand, the divergencies in the case of maltose, sucrose, raffinose and gentianose could be accounted for by the assumption that a shifting of a ring takes place during the methylation of these sugars. The writer did not publish these various considerations because he was disinclined to dispute the generally accepted assumption of ring stability during methylation in the absence of definite experimental proof of its invalidity. Instead, there was sought some way by which this hypothesis could be tested decisively. This has now been done and the results, which are shown in the preceding article,[3] prove that ring shifting is a frequent occurrence

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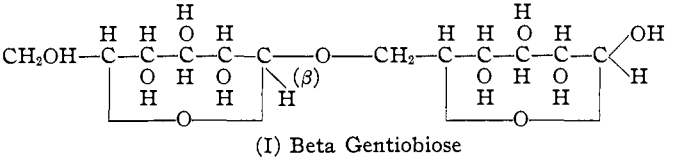
[2] Hudson, THIS JOURNAL, **48,** 1434 (1926).

[3] Hudson, *ibid.*, **52,** 1680 (1930).

during the methylation of glycosides. This disclosure, some of the fruits of which are shown in the preceding article, makes it necessary to reconsider all the structures of the compound sugars, especially the ring assignments, that have become current in recent years. This task will now be undertaken for most of the compound sugars; the results may be stated briefly to be that certain of the results from methylation studies agree with the isorotation rules, indicating ring stability during the methylation of 1,5-ring glycosides and that certain other results from methylation studies can only be reconciled with the rules by the view that a 1,3- or a 1,4-ring glycoside can shift during the methylation of some types of sugars. In the case of maltose, sucrose, raffinose, gentianose and melibiose the rules indicate structures that are different from those now generally accepted.

2. The Structure and Configuration of Gentiobiose.—The writer regards the synthesis of this disaccharide by Helferich[4] and his collaborators as conclusively establishing the disaccharide union at Carbon 6 of the primary[5] glucose molecule. The primary ring of the known crystalline gentiobiose must be the same as that in α- and β-methylgentiobioside; this was proved by Hudson and Johnson from rotatory relations.[6] The fact that Helferich synthesized α-methylgentiobioside from normal α-methylglucoside, for which the 1,B = 1,5 ring was proved in the preceding article, shows that the primary ring in the substances cited is 1,B = 1,5. Helferich's use of acetobromoglucose in the synthesis of gentiobiose is very strong evidence that the second glucose component carries a 1,5-ring and is a beta form, because normal β-methylglucoside (1,5) results from this Koenigs–Knorr synthesis when acetobromoglucose unites with methyl alcohol. The β-glycosidic structure of gentiobiose is further supported from the known hydrolysis and also the synthesis of gentiobiose by emulsin.[7] The beta crystalline form of gentiobiose is therefore 6-β-*d*-glucosido-(1,5)-β-*d*-glucose-(1,5) and its configurational formula is (I).



(I) Beta Gentiobiose

The observed initial rotation of β-gentiobiose ($[\alpha]_D$ −11) indicates that the rotation of α-gentiobiose of the same 1,5 primary ring structure may be

[4] Helferich, Bäuerlein and Wiegand, *Ann.*, **447**, 27 (1926).

[5] For brevity the reducing component of a reducing disaccharide will be designated as the primary monosaccharide and its ring as the primary ring; the glycosidic component of the disaccharide will be designated the secondary sugar and its ring the secondary ring.

[6] Hudson and Johnson, THIS JOURNAL, **37**, 1270 (1915).

[7] Bourquelot, Hérissey and Coirre, *Compt. rend.*, **157**, 732 (1913).

expected to be about 44. Bourquelot and Hérissey[8,9] have crystallized a form of gentiobiose with 2 moles of methyl alcohol of crystallization, the initial rotation of which is near 18 for the gentiobiose component. The great divergence of this value from the calculation suggests to the writer that either (1) the experimental measurement or purity of the substance may be questionable or (2) that the substance possesses a primary ring other than 1,5. Following the second alternative, let the rotation of α-gentiobiose of the 1,4 primary ring be calculated by the rules of isorotation. The molecular rotation of the known α-*d*-glucose (1,5) is 20,300, that of the unknown α-*d*-glucose[2] (1,4) is 11,900 and the difference is 8400 or in the $[\alpha]_D$ value of gentiobiose $8400/342 = 25$. If this be subtracted from the calculated rotation of α-gentiobiose of the 1,5-primary ring (44), there is obtained for the α-gentiobiose of the 1,4 primary ring $[\alpha]_D$ 19. The nearness of this value to the rotation observed by Bourquelot and Hérissey (18) is an indication that their substance may possess the 1,4 primary ring. The result is only an indication, however, not a proof, and the question must await further experimental studies before decision can be reached; it is quite possible that a repetition of the preparation may give an initial rotation near 44 rather than 19.

3. The Structure and Configuration of Trehalose.—The writer expressed the view fourteen years ago that the high dextrorotations of trehalose and its octa-acetate indicate that this disaccharide is composed of two molecules of α-*d*-glucose.[9] Today our knowledge of ring structures of the 1,4 and 1,5 type in the glucose series permits a more specific study of this question by the same considerations that were used formerly. There are four possible combinations in the trehalose group whose rotations can be calculated. The results are shown in Table I; the data for the calculations

TABLE I

CALCULATED ROTATIONS OF SOME SUGARS OF THE TREHALOSE GROUP

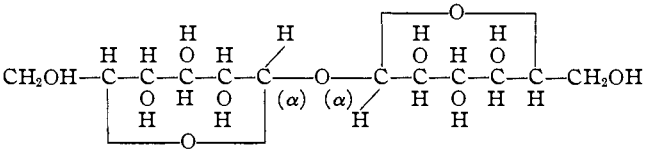
Substance	$[M]_D$ calcd.	$[\alpha]_D$ calcd.
α-*d*-Glucose (1,4) < > β-*d*-glucose (1,4)	11,880 − 5040 = 6840	20
α-*d*-Glucose (1,5) < > β-*d*-glucose (1,5)	20,300 + 3420 = 23,720	69
α-*d*-Glucose (1,4) < > β-*d*-glucose (1,5)	11,880 + 3420 = 15,300	45
α-*d*-Glucose (1,5) < > β-*d*-glucose (1,4)	20,300 − 5040 = 15,260	45

are the known rotations of the α- and β-forms of *d*-glucose (1,5), namely, $[\alpha]_D$ 113 and 19, and the calculated rotations[2] of the similar forms of *d*-glucose (1,4), namely, 66 and −28, all of which are expressed in the table as molecular rotations (m. w. 180). No one of the calculated rotations is at all near the very high dextrorotation of trehalose (197) and it is evident that the sugar is a combination of two molecules of alpha forms of *d*-glucose

[8] Bourquelot and Hérissey, *J. pharm. chim.*, **16**, 418 (1902).

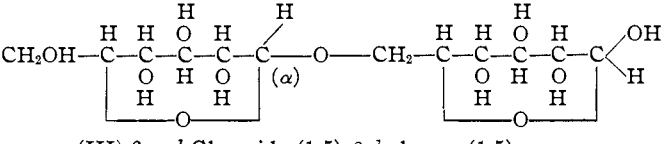
[9] Hudson, THIS JOURNAL, **38**, 1566 (1916).

but it may be any one of the three possible combinations $\alpha(1,4)<>\alpha(1,4)$, $\alpha(1,5)<>\alpha(1,5)$ and $\alpha(1,4)<>\alpha(1,5)$. Selection among these possibilities can be made by the following additional consideration. The assignment of each of these structures to trehalose gives the following values for 2A′ (see the older article)[9] in the designated order: 60,560, 43,680 and 52,100. It will be shown in the subsequent parts of the present article that one of these values (43,680), and only this one, fits in with the rotations of gentiobiose, melibiose, cellobiose and maltose to yield a uniform simple system which correlates the rotations and structures of the compound sugars by the rules of isorotation. The rotation of the octa-acetate of trehalose also fits in with the rotations of the octa-acetates of the four named sugars and one other (paragraph 8) only on the same assumption of structure. These independent results show that the full configuration of trehalose corresponds with (II) and that the sugar is α-*d*-glucose (1,5)<>α-*d*-glucose (1,5).



(II) Trehalose

4. Calculation of the Rotation of the Unknown Disaccharide 6-α-*d*-Glucosido-(1,5)-β-*d*-glucose (1,5).—This sugar, which is now unknown, differs from β-gentiobiose solely in being an alpha glucoside where β-gentiobiose is a beta glucoside. Its configuration is shown in (III).



(III) 6-α-*d*-Glucosido (1,5)-β-*d*-glucose (1,5)

The rotations of the two sugars differ because in β-gentiobiose the value for Carbon 1 of the secondary glucose is to be written $-A'$ whereas in the unknown sugar it becomes $+A'$; the remaining asymmetric carbons of the two sugars are to be assigned the same summation value B' by the optical superposition rule. We then have for the molecular rotations: β-gentiobiose, $[M]_D = B' - A' = -3800$; 6-α-*d*-glucosido(1,5)-β-*d*-glucose-(1,5), $[M]_D = B' + A' = X$. Let it now be assumed that the value of A' is constant among the compound sugars; it is obtainable[10] from the rotations of trehalose and the known forms of glucose as $A' = 21{,}840$ and hence $X = +39{,}880$. The $[\alpha]_D$ value of the beta form of this unknown sugar is

[10] Ref. 9 and paragraph 3 of the present article.

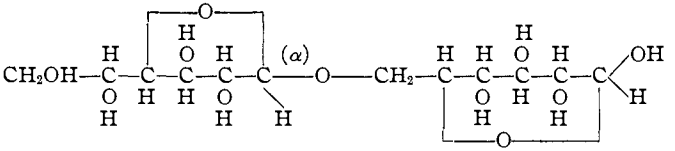
thus 39,880/342 = +117 and the rotation of its alpha form is $117 + 2a_{OH}/342 = +166$.[11]

5. The Structure and Configuration of Melibiose.—The recent methylation studies of Haworth and his collaborators[12] indicate that the 6-union is present in melibiose; the writer accepts this particular methylation datum because any ring shifting during methylation could not affect the result as regards the 6-union. Proof that the primary ring of crystalline β-melibiose (124) is of the 1,5 type is obtained through the writer's observation[9] that the rotation of this disaccharide may be accurately calculated from that of raffinose by the constants that are derivable from the rotations of β-gentiobiose (1,5 primary) and gentianose or of β-glucose (1,5) and sucrose. The presence of an alpha rather than a beta galactosidic residue in melibiose is shown by the failure of emulsin to hydrolyze the sugar; it is also indicated in a rough way by the high dextrorotation of melibiose and in a precise quantitative way by the results of the calculations which will appear in the course of the present discussion. Let us now assume that the secondary ring of melibiose is of the same type as that which exists in alpha methylgalactoside, namely, the 1,4-ring, as shown in the preceding article; the structure of beta melibiose is thus postulated to be 6-α-*d*-galactosido(1,4)-β-*d*-glucose(1,5). The rotation that is to be expected for a sugar of this structure will now be calculated from the rotations of known substances by application of the isorotation rules. The calculation starts with the rotation of beta gentiobiose, the complete structure of which has been established, and proceeds first to the calculation of the rotation of 6-α-*d*-glucosido(1,5)-β-*d*-glucose(1,5), as shown in paragraph 4. The rotation of β-melibiose is then obtainable from that of 6-α-*d*-glucosido(1,5)-β-*d*-glucose(1,5) by adding the value $Ga - G$,[9] obtainable from the molecular rotations of α-methyl-*d*-galactoside(1,4) (37,380) and α-methyl-*d*-glucoside(1,5) (30,630) as 6750 or 20° in the $[\alpha]_D$ value of a di-hexose. The calculated rotation of β-melibiose is thus $[\alpha]_D = 117 + 20 = 137$, which is near the observed value (124). The difference (13) is not large enough to indicate a different ring structure from that assumed, especially in view of the result that will next be shown from a similar calculation of the rotation of the beta octa-acetate of melibiose. Starting with the known β-gentiobiose (1,5 primary) octa-acetate ($M = -3590$), the addition of $2A'' = 69{,}320$ from trehalose octa-acetate[9] and of 1810 from the difference between the rotations of the tetra-acetates of β-methylgalactoside and β-methylglucoside, gives for β-melibiose((1,5) primary) octa-acetate $[\alpha]_D$ 100, in close agreement with the rotation (102.5) of the

[11] Hudson and Yanovsky, THIS JOURNAL, **39**, 1013 (1917); $2a_{OH} = 16{,}900$, from the molecular rotations of alpha and beta glucose.

[12] Haworth, Hirst and Ruell, *J. Chem. Soc.*, 3125 (1923); Charlton, Haworth and Hickinbottom, *ibid.*, 1527 (1927); Haworth, Loach and Long, *ibid.*, 3146 (1927).

known melibiose octa-acetate. The isorotation rules thus indicate that the full structure of β-melibiose is (IV).



(IV) Beta Melibiose

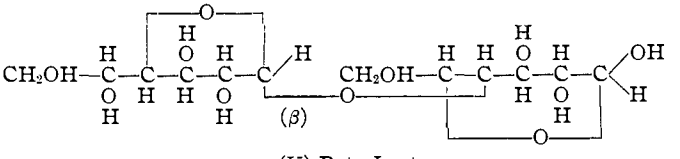
6. The Structures and Configurations of Lactose and Cellobiose.— Accepting the proof of Zemplén that the disaccharide union in these sugars is at Carbon 4 of the primary glucose molecule, it is evident that the sugars cannot possess the 1,4 primary ring and hence the 1,5-ring is assigned to them. Their low dextrorotations and their hydrolysis by emulsin indicate that they are beta glycosides. The secondary rings can be allocated by the following considerations. The various values of the molecular difference between an α-methylgalactoside and glucoside of the 1,4- and 1,5-ring structures are shown in Table II from the data of the preceding article.

TABLE II

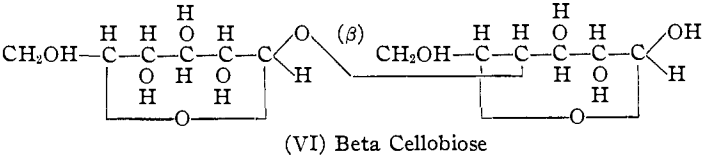
MOLECULAR DIFFERENCE OF ROTATION FOR VARIOUS PAIRS OF METHYL GLYCOSIDES

α-Methylgalactoside (1,4)	37,380	α-Methylgalactoside (1,5)	45,930
α-Methylglucoside (1,5)	30,830	α-Methylglucoside (1,4)	22,330
	6,550		23,600
α-Methylgalactoside (1,4)	37,380	α-Methylgalactoside (1,5)	45,930
α-Methylglucoside (1,4)	22,330	α-Methylglucoside (1,5)	30,830
	15,050		15,100

The value of the molecular difference in the case of β-lactose and β-cellobiose[9] is (35 −16) 342 = 6498, and it is seen that this value agrees closely with one of the differences shown in the table but is far removed from the other three. From this good evidence the writer allocates the 1,4 secondary ring to lactose and the 1,5 secondary ring to cellobiose, and the full configurations of the sugars are (V) and VI).

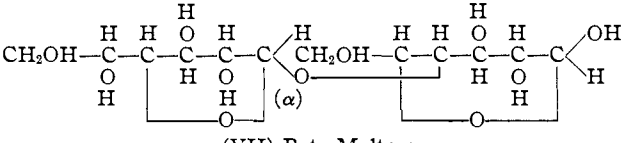


(V) Beta Lactose



(VI) Beta Cellobiose

7. The Structure and Configuration of Maltose.—It was shown in the preceding article that the researches of Fischer and Armstrong in union with the recent work of Zemplén indicates that the primary ring of β-methylmaltoside is 1,5. The results of the writer from rotatory relations[13] show that β-maltose possesses the same primary ring as β-methylmaltoside. Zemplén has proved that the disaccharide union in maltose is at Carbon 4 of the primary glucose molecule. The high dextrorotation of maltose and the fact that emulsin does not hydrolyze it show that it is an alpha glycoside. Its secondary ring can be allocated by the following considerations. Starting with the molecular rotation of β-cellobiose (5470) and adding the value of $2A' = 43{,}680$ from the rotation of trehalose (see paragraph 3), the rotation of the hypothetical 4-α-glucosido(1,5)-β-*d*-glucose(1,5) is calculated from the same considerations that were described in paragraph 4 to be $[\alpha]_D = (5470 + 43{,}680)/342 = 144$. This value is so much greater than that observed for β-maltose (118) that the writer concludes that the 1,5 secondary ring cannot be possessed by maltose. On the other hand, it can be shown that this ring is of the 1,4 type. The difference between the molecular rotations of the α-*d*-glucoses of the 1,5- and 1,4-ring types, respectively, is 20,300 − 11,880 = 8420 from Table I. Subtracting this quantity from the rotation just calculated gives $[\alpha]_D = (5470 + 43{,}680 - 8420)/342 = 119$ as the rotation of 4-α-*d*-glucosido(1,4)-β-*d*-glucose-(1,5). This rotation agrees so closely with that of β-maltose (118) that the 1,4 secondary ring may be assigned to the sugar and its full configuration becomes (VII).



(VII) Beta Maltose

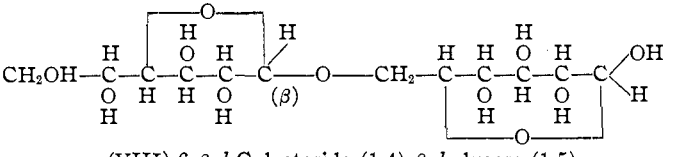
This novel result is confirmed by the similar consideration of the rotation of β-maltose octa-acetate. Adding $2A''$ (69,320, from trehalose octa-acetate)[9] to the rotation of β-cellobiose octa-acetate (−9900) gives $[\alpha]_D = (-9900 + 69{,}320)/678 = 88$ for the rotation of the beta octa-acetate of 4-α-*d*-glucosido(1,5)-*d*-glucose(1,5); this value is much greater than that observed for β-maltose octa-acetate (62.7). On the other hand, the value calculated for the beta octa-acetate of 4-α-*d*-glucosido(1,4)-*d*-glucose(1,5) is $[\alpha]_D = (-9900 + 69{,}320 - 18{,}462)/678 = 60$, which agrees closely with the rotation of β-maltose octa-acetate.[14] These two independent proofs of the 1,4 secondary ring structure of maltose can be reconciled with

[13] Hudson, THIS JOURNAL, **47**, 268 (1925); *Scientific Papers of the U. S. Bureau of Standards*, No. 533, pp. 363–364 (1926).

[14] The value 18,462 is the difference between the molecular rotations of the α-methylglucoside tetra-acetates of the 1,5- and 1,4-rings, respectively (Ref. 2).

the agreeing methylation results of Irvine and of Haworth, who obtained normal tetra-methylglucose(1,5) by the hydrolysis of fully methylated maltose, only on the view that the secondary 1,4-ring shifts to the 1,5-position during methylation. This new structure for maltose opens a wide field for speculation and experimental research. Maltose and cellobiose are not structurally similar sugars, as has been postulated during recent years, and it may consequently be that starch and cellulose differ profoundly in structure. The validity of methylation evidence in studying the structures of both these polysaccharides can no longer be regarded as secure because of the ring shifting that has been shown in the case of the secondary 1,4-ring of maltose. In the field of enzyme studies one sees that α-glucosidase and maltase hydrolyze different ring types of *d*-glucosides, the former attacking 1,5-ring α-glucosides and the latter an α-glucoside of the 1,4-ring.

8. The Structure and Configuration of the Synthetic Disaccharide, 6-β-*d*-Galactosido-*d*-glucose, of Helferich and Rauch.—These workers[15] have synthesized the octa-acetate of a new disaccharide for which they have proved the structure 6-β-*d*-galactosido-β-*d*-glucose octa-acetate, leaving only the two rings to be allocated. The primary ring is 1,5 because Helferich's synthesis of β-gentiobiose octa-acetate by like reactions in which acetobromoglucose was used in place of acetobromogalactose proves that the tetra-acetylglucose common to both syntheses possesses a 1,5-ring and is a beta form. Acetobromogalactose yields with methyl alcohol in the Koenigs–Knorr synthesis the beta methylgalactoside to which the 1,4-ring was assigned in the preceding article and the writer assumes that the galactose component of the new disaccharide therefore possesses the same 1,4-ring. It thus results that we have in the beta octa-acetates of melibiose and the new sugar a pair of known substances from which a value of $2A''$ can be obtained and it can be determined whether it agrees with the value obtained from trehalose octa-acetate ($2A'' = 69{,}320$); the molecular rotation of beta melibiose octa-acetate is 69,500 and that reported by Helferich and Rauch for the beta octa-acetate of their new disaccharide is zero, hence $2A'' = 69{,}500$ for this pair. The agreement is excellent and the full structure and configuration of the new sugar in the form present in its beta octa-acetate is thus shown to be (VIII).



(VIII) 6-β-*d*-Galactosido (1,4)-β-*d*-glucose (1,5)

[15] Helferich and Rauch, *Ber.*, **59**, 2655 (1926). Dr. Helferich informs the writer that the solvent in which the rotation of the octa-acetate was measured and found to be zero was chloroform, which is not stated in the original article, and that a recent repetition of the measurement has given the same result.

9. Proof of the Invalidity of Haworth's[16] Application of the Isorotation Rules to Maltose and Cellobiose.—Haworth obtains from beta cellobiose and beta maltose, through the assignment of a 1,5-secondary ring to maltose, the value $2A' = (118 - 16)342 = 34,884$, and for the beta octa-acetates of these sugars the value $2A'' = 52,400$. It readily can be shown that these values are so low that they must be rejected as impossible ones, and the evidence is precisely that which Haworth has advanced in proof of their correctness. He shows that the value of $2A$ from the alpha and beta methylglucosides or galactosides is about 37,460, and that the similar value for the tetra-acetates of these substances is about 53,500 and claims that the approximate agreement thus shown with the values from the maltose–cellobiose series proves that his ring assignments are correct. The error in the argument lies in his overlooking the well-established fact[17] that the rotation of Carbon 1 in a methyl glycoside is much lower than for glycosides with heavier groups than methyl attached; the value for $2A'$ in the disaccharides must be far larger than the $2A$ of the methylglycosides. Some recent evidence on this subject has been obtained by Pacsu[18] through the preparation of the alpha and beta forms of cyclohexanol-glucoside and their tetra-acetates. The value of $2A'$ for these glycosides is found to be 45,750 and that of $2A''$ for their acetates to be 62,560. These values are far greater than those from the methyl glycosides and their acetates and the values used by Haworth but are comparable with the higher values that the writer has accepted for the compound sugars. The explanation of the low values of Haworth is that the secondary ring of maltose is not 1,5 but rather 1,4, as shown in paragraph 7; on the methylation of maltose this ring shifts to the 1,5-position.

10. The Structure and Configuration of Sucrose.—The writer showed some years ago[19] that the hydrolysis of sucrose by invertase yields as the first detectable hexoses the form of α-*d*-glucose (113) to which the 1,5-ring is now assigned and a form of fructose, of $[\alpha]_D$ +17, which he then designated as α-fructose because of its mutarotation in the levo direction. It is a remarkable experimental fact, to which too little attention has been paid in recent years, that the nearly instantaneous hydrolysis of sucrose by very strong invertase preparations yields initially a mixture of hexoses having a mean rotation of the same value as that of sucrose. For many years this accurate observation could not be reconciled by the writer with structural considerations because if sucrose is composed of alpha forms of both glucose and fructose, its rapid enzymotic hydrolysis should result in a large initial drop in dextrorotation. Consider for example the case of the enzymotic

[16] Ref. 12, second article.

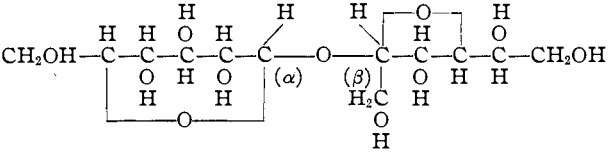
[17] Hudson, THIS JOURNAL, **31**, 82 (1909).

[18] The article by Eugen Pacsu on this subject is now in press and will appear shortly; the writer is indebted to him for kind permission to use his results.

[19] Hudson, THIS JOURNAL, **31**, 655 (1909).

hydrolysis of trehalose, of the structure α-glucose(1,5)<>α-glucose(1,5), which is assumed to be so rapid that the sugar is completely hydrolyzed before the mutarotation of the resulting α-glucose(1,5) reaches sensible proportions. The $[\alpha]_D$ value of the solution should drop on hydrolysis immediately from 197, the rotation of trehalose, to about 113, the rotation of α-glucose(1,5). In the case of the unknown β,β-trehalose, of the structure β-glucose(1,5)<>β-glucose(1,5), the rotation of which has been calculated[9] to be $[\alpha]_D$ -58, a similar enzymotic hydrolysis should cause the rotation to rise immediately from -58 to about $+19$, the rotation of β-glucose(1,5). However, in the case of the hydrolysis of the unknown α,β-trehalose (calculated $[\alpha]_D$ 70) the rotation should not change immediately because it is the average of the rotations of its constituent alpha and beta glucoses. It would thus seem that the form of fructose which is initially liberated from sucrose (and from raffinose and gentianose, which are derivatives of sucrose by the attachment of another hexose molecule to the glucose constituent of sucrose) is a beta form of the observed $[\alpha]_D$ $+17$. The alpha form of this type of fructose would necessarily be a very strongly dextrorotatory substance of $[\alpha]_D$ approximately $+111$ if the difference ($2a_{OH}$) in the fructose series is the same as for the forms of glucose. If these considerations are correct, however, how can the fact be explained that this initially liberated fructose, of $[\alpha]_D$ 17, acts like an alpha sugar by mutarotating to the strongly negative rotation (-90) of stable fructose solutions? The fructose actually behaves like an α-form, judging from its mutarotation, but like a β-form, judging from the absence of an immediate change following the nearly instantaneous hydrolysis of sucrose. The writer made these observations in 1909; it is now apparent through the knowledge that has been gained in the subsequent years concerning the occurrence of various ring types among the sugars that the measurements furnish clear proof that the initially liberated fructose is indeed a beta-form but that it shifts its ring during the mutarotation. With this point cleared up one may proceed with the study of the structure of sucrose by rotatory considerations. We start then with the view that sucrose is α-*d*-glucose-(1,5)<>β-fructose(2,?), from the evidence that has been discussed, and the sole problem remaining is to allocate the position of the fructose ring. It cannot be 2,5 because the rotation of α-*d*-glucose(1,5)<>β-*d*-fructose-(2,5) may be calculated from those of the known α-methyl-*d*-glucoside-(1,5) (30,830) and β-methyl-*d*-fructoside(2,5) ($-33,400$) to be $[\alpha]_D$ = (30,830 $-$ 33,400)/342 = -8, which is far different from the rotation of sucrose (66). Haworth has assigned this 2,5-ring structure to the fructose portion of sucrose from his methylation evidence; it is clear that this ring cannot be present and assuming the correctness of his experimental identifications of the methylated products one can only conclude that the fructose ring has shifted during methylation. Let the possibility that the

fructose ring may be 2,6 be examined; referring to the preceding article, paragraph 20, it is evident that β-methyl-*d*-fructoside(2,6) must be a more strongly levorotatory substance than β-methyl-*d*-fructoside(2,5) in analogy with the corresponding substances of the *d*-arabinose series and that consequently α-*d*-glucose(1,5) $<>$ β-*d*-fructose(2,6) will have a greater levorotation than the value $[\alpha]_D$ -8, mentioned previously. This result excludes the possibility of the 2,6-ring, and unless one assumes an ethylene oxide ring, which appears highly improbable because of instability, the assignment of the 2,4-ring becomes necessary and sucrose is α-*d*-glucose-(1,5) $<>$ β-*d*-fructose(2,4) as shown in the full configurational formula (IX).

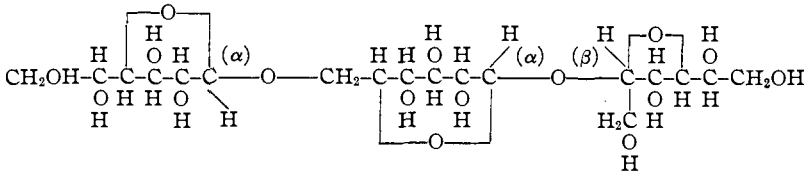


(IX) Sucrose

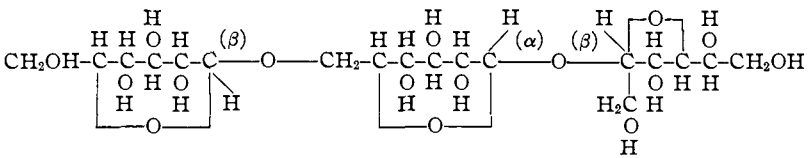
The rotation of the octa-acetate of sucrose ($[\alpha]_D$ 59.6) supports the foregoing results. The rotation of the octa-acetate of α-*d*-glucose(1,5) $<>$ β-*d*-fructose(2,5) is calculated from the rotations of the tetra-acetates of α-methyl-*d*-glucoside(1,5) ($[M]_D$ 47,300) and β-methyl-*d*-fructoside(2,5) ($-45{,}100$) to be $[\alpha]_D = (47{,}300 - 45{,}100)/678 = 3°$, which is greatly different from the value for sucrose octa-acetate. The assignment of the 2,6-ring leads to a value even more divergent and one is thus led to assign the 2,4-ring. The ease with which sucrose is hydrolyzed by acids conforms with the assigned configuration; the propylene oxide ring of its fructose component is the 1,C = 1,3[2] ring of the mannose, rhamnose and lyxose series and sucrose properly takes its place beside the very easily hydrolyzed methylmannoside, methylrhamnoside and methyllyxoside of this ring type, as was suggested in paragraph one.[20]

11. The Structures and Configurations of Raffinose and Gentianose.—The configurations that have been shown for melibiose, gentiobiose and sucrose enable one to write the following full configurations for these trisaccharides (X and XI).

[20] In a recent article Haworth, Hirst and Miller [*J. Chem. Soc.*, 2469 (1929)] attempt to support their assumption of a 1,5-ring in these easily hydrolyzed glycosides by suggesting a new type of stereoisomerism which is in conflict with the Le Bel–van't Hoff theory of the asymmetric carbon atom as heretofore applied in the sugar group; since their experimental results are readily intelligible on the view that the 1,3-ring can shift during methylation and since such shiftings of 1,3- and 1,4-rings have been demonstrated in numerous cases in this article and the preceding one, their discussion of the supposed invalidity of the Le Bel–van't Hoff theory seems to the writer to have no substantial basis.



(X) Raffinose



(XI) Gentianose

Postscript of March 4, 1930.—It will be shown in a later article that the rotations of primverose and its beta hepta-acetate agree with the structure 6-[β-*d*-xylosido(1,5)]-*d*-glucose(1,5), that the structure of vicianose is in all probability 6-[α-*l*-arabinosido(1,5)]-*d*-glucose(1,5) and that vicianin is 6-[α-*l*-arabinosido(1,5)]-β-*d*-glucosido(1,5)-*l*-mandelonitrile.

12. Summary

The occurrence of ring shifting during the methylation of some glycosides, as was shown in the preceding article, makes it necessary to determine the structures and configurations of the compound sugars by methods which avoid this disturbing complication. It is shown that the isorotation rules apply closely to the group of compound sugars and that the full structures and configurations of these substances can be determined by the use of these rules in conjunction with other data on structure that are valid whether or not ring shifts occur during methylation. The results give new structures for maltose, melibiose, sucrose, gentianose and raffinose.

WASHINGTON, D. C.

NOTES

A Note on the Preparation of Lecithin.—The preparation of pure lecithin was recently undertaken in order to study the relation of the pure product to the process of blood coagulation.[1]

The method used was that described by Levene and Rolf;[2] a simplification of similar methods previously used by Bergell,[3] McLean[4] and by Levene[5] and his co-workers. It consists in the simple extraction of dried

[1] A. Wadsworth, F. Maltaner and E. Maltaner, *Am. J. Physiol.*, **91**, 423 (1930).

[2] P. A. Levene and I. P. Rolf, *J. Biol. Chem.*, **72**, 587 (1927).

[3] P. Bergell, *Ber.*, **33**, 2584 (1900).

[4] H. McLean, *Biochem. J.*, **9**, 351 (1915).

[5] P. A. Levene and C. J. West, *J. Biol. Chem.*, **34**, 175 (1918); P. A. Levene and I. P. Rolf, *ibid.*, **46**, 353 (1921).