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The Conformations of the Disaccharides, Cellobiose and Maltose[1]

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The oxidation of β-cellobiose by bromine in buffered aqueous solution at 0° is more rapid than that of the α-anomer (k_β/k_α = 56.5). Both methyl β-cellobioside and methyl β-maltoside form levorotatory complexes with a standard cuprammonium solution. The decreased optical rotation of methyl β-maltoside in alkali led to an examination of other glycosides; methyl α- and β-D-arabinopyranoside, β-D-galactopyranoside, α- and β-D-idopyranoside and α-D-mannopyranoside were also found to be alkali labile. *N* sodium hydroxide however, has only a small influence on the rotation of methyl β-maltoside in cuprammonium solution. The possible conformations of the reducing and non-reducing glucose units in cellobiose and maltose are discussed in the light of these results and information already in the literature. It is concluded that in cellobiose both glucose units have the C 1 conformation. In maltose, the reducing glucose unit is probably C 1; the non-reducing unit may be in or near a "skew" conformation lying intermediate between the B1 and 3 B boat forms.

Although the conformations of the pentose and hexose sugars are now well established, there is little information relating to oligo- and polysaccharides. Reeves[2] pointed out difficulties in making molecular models containing α-1,4-linked glucose units and concluded that amylose contained more than one ring conformation. Since the evidence pointed to two boat forms in amylose, it was of interest to attempt a determination of the ring conformations in the simpler disaccharides, cellobiose and maltose.

Experimental

Methyl β-cellobioside (hydrate, m.p. 135–136°) was prepared by catalytic barium deacetylation of methyl heptaacetyl β-cellobioside[3]; methyl β-maltoside (hydrate, m.p. 111°) was prepared by the method of Schock, Wilson and Hudson.[4] All optical rotations were determined at 24 ± 2° using the sodium-D line, or the mercury line at 436 mμ isolated with an interference filter. The standard cuprammonium solution, cupra B, was that described by Reeves.[5] Rotations in cupra B were determined in a 50 mm. polarimeter tube, fitted with central tubulature and screw ends.[6]

Oxidation experiments with cellobiose were carried out by adding finely powdered cellobiose (about 100 mg.) or an ice-cold equilibrium solution containing the same amount in 2 ml. of water, to 50 ml. of a saturated solution of bromine in 0.5 *M* phosphate buffer, *p*H 5.5. For oxidation of the solid cellobiose, 2 ml. of ice-cold water was also added. The reaction flask was well cooled in ice, and the solution was stirred mechanically. A slow stream of nitrogen carrying bromine vapor was bubbled through the solution to maintain the bromine saturation. At definite time intervals, 2-ml. samples were withdrawn and immediately shaken with a solution of corn oil in chloroform (1:1) to remove bromine. After centrifugation and filtration, aliquots of the clear, aqueous solution were diluted 1:5 or 1:10 and analyzed for cellobiose using the sugar assay of Folin and Malmros.[7] Prior to analysis, the solutions were titrated with 0.1 *N* NaOH to the phenolphthalein end-point; standard cellobiose solutions were prepared containing the same amount of a bromine–phosphate buffer solution treated with corn oil, as in the oxidation samples. There were only small changes in the *p*H of the oxidation solutions during the reaction; in a typical case, the change was from *p*H 5.5 to 5.3 after 30 minutes and to *p*H 5.1 after 60 minutes. For determination of bromine concentrations, 1-ml. samples of the oxidation mixture were withdrawn and added to 10% potassium iodide. The liberated iodine was titrated with 0.1 *N* thiosulfate.

Results and Discussion

Conformation of the Reducing Glucose Units.—As shown in Table I, the oxidation of solid β-cellobiose with bromine water buffered at *p*H 5.5 was rapid. About 90% was oxidized in 10 minutes. With the equilibrium solution, there was an initial rapid oxidation, and 63% of the total sugar was oxidized in 10 minutes. A less reactive component was then oxidized very slowly, and at the end of 2 hr. only 78.8% of the total sugar had been oxidized.

TABLE I

BROMINE OXIDATION OF CELLOBIOSE AT *p*H 5.5 AND 0°[a]

Solid β-cellobiose			Cellobiose equilibrium soln.		
Time, min.	Sugar concn., μg./ml.	Oxidized, %	Time, min.	Sugar concn., μg./ml.	Oxidized, %
0	1925	0.0	0	1925	0.0
0.9	1845	4.1	0.8	1440	25.1
1.5	1652	14.2	3	1030	46.4
2	1417	26.4	6	815	57.7
3	1089	43.4	9	740	61.6
4	830	56.9	15	690	64.1
5	590	69.4	30	610	68.3
6	447	76.9	47	540	71.9
8	276	85.6	60	530	72.6
10	187	90.4	120	410	78.8
12	140	92.7			

[a] Conditions as in text. Even though finely powdered solid cellobiose was used, there was a definite time lag before solution was complete. If the results are plotted as log $(A/(A - X))$ against time, the straight line obtained does not pass through the origin and intersects the time axis at 0.9 minute. For this reason, the rate of oxidation of the solid cellobiose over the first few minutes appears to be somewhat slower than that of the equilibrium solution.

The results from the equilibrium solution were plotted as log % cellobiose unoxidized against time. Extrapolation to zero time of the line for the slow oxidation by the method of Isbell and Pigman[8] indicated that the equilibrium solution contained 37.2% of the less reactive α-anomer. Values of $1/t \log (A/(A - X))$, where A is the amount of sugar present initially, and $A - X$ the amount at time t, were calculated as described by Isbell and Pigman.[8,9] For solid β-cellobiose, the value

(1) Presented in part before the Carbohydrate Division at the 132nd meeting of the American Chemical Society, New York, September, 1957. This work was supported in part by a Research Grant (A-725) from the National Institutes of Health, United States Public Health Service.

(2) R. E. Reeves, THIS JOURNAL, **76**, 4595 (1954).

(3) E. Pacsu, *ibid.*, **52**, 2571 (1930).

(4) T. J. Schock, E. J. Wilson and C. S. Hudson, *ibid.*, **64**, 2871 (1942).

(5) R. E. Reeves, *Advances in Carbohydrate Chem.*, **6**, 107 (1951).

(6) The 50 mm. polarimeter tube was obtained from O. C. Rudolph and Sons, Caldwell, N. J.

(7) O. Folin and H. Malmros, *J. Biol. Chem.*, **83**, 115 (1929).

(8) H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, **10**, 337 (1932).

(9) H. S. Isbell and W. W. Pigman, *ibid.*, **18**, 141 (1937).

was 120.2 × 10^{-3}; for α-cellobiose, present in equilibrium solution, the value was 3.08 × 10^{-3} (decimal logarithms, reciprocal minutes). Since the average bromine concentrations in the experiments with solid and equilibrium cellobiose were respectively 0.146 and 0.210 moles per liter, the velocity constants, calculated by the method of Isbell and Pigman, are k_β = 823.3 × 10^{-3} and k_α = 14.57 × 10^{-3}. The rate ratio, k_β/k_α, is therefore 56.5. Isbell and Pigman[9] had observed earlier that the oxidation rates of the β-anomers of maltose and lactose were also more rapid than those of the α-forms. The velocity constants for these disaccharides and for cellobiose are similiar to those of glucose (Table II).

TABLE II

BROMINE OXIDATIONS OF GLUCOSE DERIVATIVES[a]

Sugar	Velocity constant (decimal logarithms, reciprocal min.) $k_\beta \times 10^3$	$k_\alpha \times 10^3$	Rate ratio, k_β/k_α	Composition of equilibrium soln. α, %	β, %
Glucose	1255	27.5	45.6	37.4	62.6
Lactose	952	20.9	45.5	37.5	62.5
Maltose	1528	23.7	64.5	37.7	62.3
Cellobiose	823	14.6	56.5	37.2	62.7

[a] Values for glucose, lactose and maltose ($BaBr_2$–$BaCO_3$ buffer) are from Isbell and Pigman, footnote 9. In each case, the values for k_β are those from measurements with solid β-anomer; values for k_α from measurements with equilibrium solution.

In halogen oxidations of pyranoses and methyl pyranosides it is usually, but not always, the case that β-anomers are the more rapidly oxidized; however, there are apparently no exceptions to the statement that regardless of any nomenclatural difficulties, the more rapidly oxidized anomer of a pyranose of stable conformation has an equatorial OH or OCH_3 group.[10] The oxidation experiments therefore lead to the conclusion that in both β-cellobiose and β-maltose, the anomeric OH group is equatorial. Further, the general similarity of the oxidation rates of the anomers of glucose and of the disaccharides, together with the almost identical compositions of the equilibrium solutions (Table II), suggest identical conformations for the reducing glucose units in each case, *i.e.*, C 1. β-Maltose and β-cellobiose both mutarotate in an upward direction and the β-anomers of their derivatives are less dextrorotatory than the α-forms. A C 1 conformation for the reducing glucose units is therefore also indicated by the following generalization, previously proposed by the writer[10]—"For any pyranose of stable C 1 conformation, regardless of membership in the D- or L-configurational series, the more dextrorotatory member of the anomeric pair will have the anomeric substituent in the axial position. The anomer with the glycosidic substituent in the axial position will in general be the least reactive." A similar conclusion has been reached by Reeves[11] who has utilized the optical rotation of the recently described methyl α-maltoside[12] in applying Hudson's First Rule of Isorotation. A 2 A value of 34,180 is obtained for the partial rotation of carbon 1 in maltose, which is in good agreement with the value of 37,500 calculated for the α- and β-anomers of methyl glucopyranoside.

The anomeric OH group of the reducing β-glucose unit in C 1 conformation is equatorial for maltose and cellobiose, as required by the results of the bromine oxidations. The only other conformations placing the anomeric OH group in an equatorial position are B 2, B 3 and 1 B. (For the moment, only the two chair and six boat conformations of Reeves[5] will be considered.) Examination of models strongly suggests that the preferred 1,4-glycosidic link must involve equatorial bonds in both glucose units. Reeves[2] pointed out the difficulty of forming disaccharides from glucose units in C 1 conformation through an α-1,4-linkage, where an equatorial and an axial bond are present. Similar considerations apply to the linkage with two axial bonds. Assuming therefore, that both the α- and β-1,4-linkages require equatorial bonds, the only conformations permissible for the reducing glucose unit in maltose and cellobiose must have equatorial OH groups both at C_1 and C_4. Of the possibilities just considered only the C 1 conformation meets this requirement.

Conformations of the Non-reducing Glucose Units.—With the β-1,4-disaccharide, cellobiose, a C 1 conformation for the non-reducing glucose unit would give the necessary alignment of the C_1–OH bond. Since the glucopyranose ring assumes the most stable C 1 conformation wherever possible, cellobiose is considered to contain two C 1 glucose units with all substituent groups in the preferred equatorial positions. This arrangement is confirmed by an incomplete X-ray diffraction study of crystalline cellobiose.[13] The requirement for an equatorial arrangement of the α-C_1–OH in the non-reducing glucose unit of maltose limits the possible conformations to 1 C or those occurring in that portion of the flexible cycle described by 2 B ⇄ B 1 ⇄ 3 B. These forms are inherently less stable than C 1 for the D-glucose configuration. The non-reducing glucose unit in maltose should therefore be less stable than the same unit in cellobiose and the reducing glucose in maltose itself. Two types of reaction confirm this predicted instability. (a) With the exception of the conformationally unstable gulose, β-anomers of methyl glycopyranosides are the more readily hydrolyzed.[14] However, the β-glucoside, cellobiose, is hydrolyzed more slowly than the α-glucoside, maltose ($k_{\text{cellobiose}}/k_{\text{maltose}} = 0.35$).[15] Since in these hydrolyses the bond broken is that between oxygen and the anomeric carbon,[16] the reversed order of reactivity observed with maltose and cellobiose is probably associated with instability in the non-reducing glucose unit of mal-

(10) R. Bentley, THIS JOURNAL, **79**, 1720 (1957).
(11) R. E. Reeves, *Ann. Rev. Biochem.*, **27**, 15 (1958).
(12) S. Peat, W. J. Whelan and G. Jones, *J. Chem. Soc.*, 2490 (1957).
(13) J. Wunderlich, *Compt. rend.*, **240**, 1350 (1955).
(14) R. Bentley, *Nature*, **176**, 870 (1955); J. T. Edward, *Chemistry & Industry*, 1102 (1955); A. B. Foster and W. G. Overend, *ibid.*, 566 (1955); J. Schreiber and A. Eschenmoser, *Helv. Chim. Acta*, **38**, 1529 (1955).
(15) The hydrolytic rates are taken from W. W. Pigman and R. M. Goepp, "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y., 1948, p. 203–206 and 437.
(16) C. A. Bunton, T. A. Lewis, D. R. Llewellyn, H. Tristram and C. A. Vernon, *Nature*, **174**, 560 (1954).

TABLE III

OPTICAL ROTATIONS OF METHYL DISACCHARIDES IN WATER AND CUPRA B

Compound[a]	$[\alpha]^{24}$ in water D-line, deg.	$[\alpha]^{24}$ in water 436 mμ, deg.	$[\alpha]^{24}$ 436 in cupra B, deg.	Rotational shift,[b] deg.
Methyl β-maltoside	+83	+152	− 88	− 854
Methyl β-cellobioside	−19	− 37	−400	−1294

[a] Concentration in each case = 1%. [b] Rotational shift = ($[\alpha]^{24}$ 436 in cupra B − $[\alpha]^{24}$ 436 in H_2O) × mol. wt./100.

shifts (−1,640 and −1,294°, respectively), and such differences as do exist may be reasonably explained as due to the 3,4-complex being slightly favored over the 2,3-complex in the non-reducing glucose unit. For methyl β-maltoside the observed value of −854° is not in good agreement with any of the calculated values. The calculated shift with the non-reducing glucose unit in B 1 conformation is +160°, and for the same unit in 3 B conformation, the value is −1,640°.

TABLE IV

CALCULATED ROTATIONAL SHIFTS FOR METHYL DISACCHARIDES IN CUPRA B

Conformation of non-reducing glucose unit	Configuration of OH groups C_1	C_3	C_4	Projected angle between OH groups[a] 2:3 deg.	3:4 deg.	Possible complex[b]	Rotational shift for non-reducing glucose unit,[c] deg.	$S_R + S_{N.R.}$,[d] deg.
C 1	e	e	e	− 60	+ 60	Compensating	+ 350	−1640
B 1	a	e	e	−120	+ 60	Dextro at 3:4	+2150	+ 160
1 C	a	a	a	+180	+120	None	0	−1990
2 B	a	a	e	+180	+120	None	0	−1990
3 B	e	e	e	− 60	+ 60	Compensating	+ 350	−1640

[a] Projected angle as defined by R. E. Reeves, THIS JOURNAL, 71, 212 (1949). [b] For definitions see R. E. Reeves, *ibid.*, 71, 215 (1949). [c] Average values calculated from Reeves' results with methyl substituted methyl glucopyranosides (footnote 6) and methyl α- and β-D-6-desoxyglucopyranosides (R. E. Reeves, *ibid.*, 72, 1499 (1950). [d] S_R = rotational shift for reducing C 1 glucose unit (taken as −1990°); $S_{N.R.}$ = rotational shift for non-reducing glucose unit.

tose.[17] The validity of such a comparison is supported by comparison of the rates of hydrolysis of lactose and cellobiose, and methyl β-galactoside and methyl β-glucoside. The β-galactoside, lactose, is more rapidly hydrolyzed than the β-glucoside, cellobiose ($k_{lactose}/k_{cellobiose}$ = 2.8); similarly, methyl β-galactoside is more readily hydrolyzed than methyl β-glucoside ($k_{methyl\ \beta\text{-galactoside}}/k_{methyl\ \beta\text{-glucoside}}$ = 4.8). (b) A similar situation is found in periodate oxidation experiments. At 15–20°, methyl α-glucoside and methyl β-cellobioside form 1 mole of formic acid at almost identical rates; 300 hr. for the glucoside and 291 hr. for the cellobioside. With methyl β-maltoside, the same reaction takes only 163 hr.[18]

Since a consideration of the instability factors associated with the possible conformations provided no clear evidence, the optical rotations of methyl β-maltoside and methyl β-cellobioside were determined in cuprammonium solution.[5] Both compounds formed complexes as shown by the observed large rotational shifts which are both levorotatory (see Table III).

Assuming that in a disaccharide the rotational shift is the sum of the observed shifts for each monosaccharide unit, theoretical values for the possibilities suggested earlier were calculated from the results of Reeves[5] (see Table IV). For methyl β-cellobioside, where both glucose units are assumed to be C 1, there is a reasonable agreement between the calculated and observed rotational

(17) An alternative suggestion by F. Shafizadeh and A. Thompson (*J. Org. Chem.*, 21, 1059 (1956)) is that a large aglycon in an axial position will tend to make an α-D-glycopyranoside more susceptible to hydrolysis than the β-anomer. As illustrations, they cite phenyl α-D-glucopyranoside, maltose and isomaltose which are hydrolyzed faster than phenyl β-D-glucopyranoside, cellobiose and gentiobiose, respectively. If the considerations developed in the present paper are correct, the glycosidic bond in maltose does not contain an axial component, and the explanation of Shafizadeh and Thompson is not applicable in this case.

(18) T. G. Halsall, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1427 (1947).

It therefore seemed possible that a conformation between these two extremes might be involved.

In this connection, it may be pointed out that Reeves[2] has suggested that the decreased optical rotation of amylose in alkaline solution results from the fact that recrystallized amylose may contain two ring conformations, whereas alkali amylose contains but one. Reeves[2] observed a similar decreased optical rotation of methyl β-maltoside (but not methyl β-cellobioside) in alkali, and Reeves and Blouin[19] have recently reported alkali sensitivity with methyl α- and β-D-galactopyranoside, methyl β-D-mannopyranoside, methyl β-D-altroside and methyl β-L-arabinopyranoside. As shown in Table V, the present work has indicated alkali sensitivity in three other compounds (methyl α-D-arabinopyranoside, methyl α- and β-D-idopyranoside) and has confirmed some of their earlier observations. Methyl α-D-mannopyranoside, reported by Reeves and Blouin as alkali stable in *N* NaOH, shows a difference of 9.3° in 5 *N* NaOH. The observed alkaline shifts have been attributed[19] to conformational changes so that OH groups, axially oriented in neutral solution, move to equatorial positions. If such conversions are possible, this factor might be important in considering measurements on alkali labile glycosides in the strongly alkaline cupra B solution (23% ammonia). The effect of solution in strong ammonia on the optical rotation of a number of glycosides was therefore examined. In each case (see Table V) the observed differences were all less than 5°. Reeves and Blouin consider a difference of greater than 7° as indicating alkali sensitivity.

Furthermore, if NaOH does cause conformational changes, these might be revealed by determination of rotational shifts in cupra B solutions containing NaOH. There was, however, only a small difference in the optical rotation of methyl β-maltoside in cupra B ($[\alpha]^{24}436$ −88°) and in

(19) R. E. Reeves and F. A. Blouin, THIS JOURNAL, 79, 2261 (1957).

TABLE V

EFFECT OF ALKALI ON OPTICAL ROTATIONS OF METHYL PYRANOSIDES

Methyl	Sp. rotn. in *M* NaCl − sp. rotn. in *N* NaOH,[a] deg.	Sp. rotn. in H_2O − sp. rotn. in 5 *N* NaOH, deg.	Sp. rotn. in H_2O − sp. rotn. in concd. NH_3, deg.
Stable			
β-D-Xyloside	1.2	1.7	...
β-Cellobioside	1.3[b]	0.7	2.1
Labile			
α-D-Arabinoside	.....	−30.5	−4.1
β-D-Arabinoside	−10.0[c]	−13.2	3.1
β-D-Galactoside	9.6	10.8	4.8
α-D-Idoside[d]	.....	8.6	...
β-D-Idoside[d]	.....	−11.4	−1.3
α-D-Mannoside	− 0.5	9.3	...
β-Maltoside	16.2[b]	12.6	0.9

[a] Results of Reeves (footnote 2) and Reeves and Blouin (footnote 19). All measurements made with the sodium-D line. [b] Values for sp. rotn. in H_2O − sp. rotn. in 5 *N* NaOH (Reeves, footnote 2). [c] Values from L-enantiomer. [d] Prepared as described by E. Sorkin and T. Reichstein, *Helv. Chim. Acta*, 28, 1 (1945).

cupra B containing *N* NaOH ($[\alpha]^{24}436$ −86°). The values for the rotational shifts ($[\alpha]^{24}436$ cupra B − $[\alpha]^{24}436$ H_2O) × mol. wt./100 and ($[\alpha]^{24}436$ cupra B, *N* NaOH − $[\alpha]^{24}436$ *N* NaOH) × mol. wt./100 are, respectively, −854 and −761°. The conformational change predicted by Reeves and Blouin[19] would be from B 1 (OH on C_2 axial) to 3 B (OH on C_2 equatorial) with a change of rotational shift from +160 (B 1) to −1,640° (3 B). The observed change is in any case small, and the rotational shift becomes slightly less rather than more negative. This result suggests that the alkali sensitivity of glycosides may not be directly related to conformational changes. An alternate possibility is that the changes reflect compound formation with alkali. The KOH compounds of maltose and cellobiose have been known for some time.[20] With cellobiose, the maximum number of KOH molecules per molecule of disaccharide is 2; they are located at C_1 in the reducing glucose unit and C_6 in the non-reducing glucose unit. With maltose on the other hand, 3 molecules of KOH may be used in compound formation, located at C_1 in the reducing glucose unit and at C_2 and C_6 in the non-reducing unit. The changed optical rotation of methyl β-maltoside in alkali could therefore be attributed to compound formation, specifically at C_2 in the non-reducing glucose unit. In any case, the C_2–OH in the non-reducing unit of maltose is clearly more acidic than that in cellobiose,[21] indicating a different orientation for this group in maltose. It is therefore concluded that the non-reducing glucose unit of maltose does not have a simple 3 B conformation, since if this were so the C_2–OH in both cellobiose and maltose would have the *same* equatorial orientation.

Although it is possible that in cupra B solution, methyl β-maltoside forms an equilibrium mixture containing the non-reducing glucose unit in both B 1 and 3 B conformations, a more likely possibility is the existence of a distinct conformation between these two extremes. Reeves[22] has pointed out that these two conformations are directly interconvertible through a "skew" conformation in which there are four ring atoms in a plane (See Fig. 1). Such a skew intermediate might have a

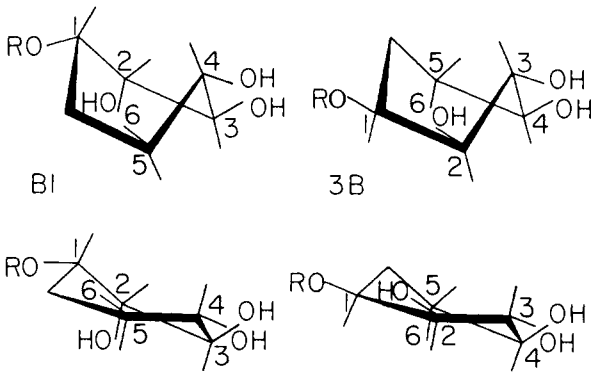

Fig. 1.—Boat and skew conformations for the non-reducing glucose unit of maltose. In each case, R is the reducing glucose unit, and for clarity the terminal CH_2OH group is represented by 6; the oxygen atom of the ring and hydrogen atoms substituted directly on ring carbon atoms have also been omitted in the drawings. Conformation B 1 is in the upper left hand corner, and 3 B in the upper right corner. The *same* skew intermediate between these two extremes is drawn in two ways to indicate the relation with the boat forms. For conformation B 1, carbon atoms 1, 2 and 3 are placed identically in the boat and skew forms, and for conformation 3 B, the same is true of the ring oxygen and carbon atoms 5 and 4. In the skew form, carbon atoms 1, 2, 3 and 5 lie in a plane.

rotational shift in cupra B between that of the two extreme boat forms. Inspection of molecular models of the skew conformation reveals that the projected angle between the C_3–OH and C_4–OH bonds remains at approximately +60° for the conformational changes B 1 ⇄ skew ⇄ 3 B. However, the angle between the C_2–OH and C_3–OH bonds varies from −120° ⇄ −90° ⇄ −60°, respectively. The 3,4-complex is therefore the same in both boat and skew conformations (+2,150°). With the C_2 and C_3–OH groups, the change is from no complex in B 1 conformation to formation of a levo complex (−1,993°) in 3 B conformation. In the intermediate skew conformation, the projected angle between these groups will be −90°; although there is no experimental observation of complex formation by cuprammonium with OH groups at such an angle, molecular scale models suggest that it is not impossible. There are two further points of interest in connection with the skew conformation. Although the terms axial and equatorial cannot be applied precisely to bonds of the skew form, it may be noted that (a), the glycosidic bond at C_1 of the non-reducing unit maintains an orientation which is still essentially equatorial; and (b), with respect to that plane most nearly accommodating the oxy-

(20) E. G. V. Percival, *J. Chem. Soc.*, 1160 (1934); E. G. V. Percival and G. G. Ritchie, *ibid.*, 1765 (1936); W. J. Headle and E. G. V. Percival, *ibid.*, 1690 (1938).

(21) The first and second dissociation constants of maltose have also been found to be higher than those of other sugars (glucose, lactose, sucrose) (P. Hirsch and R. Schlags, *Z. physiol. Chem.*, **141A**, 387 (1929)).

(22) R. E. Reeves, presented before the Carbohydrate Division at the 132nd meeting of the American Chemical Society, New York, September, 1957. See also footnote 11.

gen and carbon atoms of the pyranose ring, the CH_2OH group is somewhat less axial than in the pure 3 B conformation. This advantage is most clearly illustrated by the drawing of the skew form below that of conformation B 1 in Fig. 1.

In summary, it appears likely that the conformation of the non-reducing glucose unit is not one of the well known boat or chair forms. The skew conformation which is an intermediate in going from B 1 to 3 B meets all of the experimental and theoretical requirements discussed in this paper.

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[CONTRIBUTION FROM THE ROBERT W. LOVETT MEMORIAL UNIT, MEDICAL SERVICES OF THE MASSACHUSETTS GENERAL HOSPITAL, AND FROM THE DEPARTMENT OF MEDICINE, HARVARD MEDICAL SCHOOL]

The Synthesis of 2-Amino-1,6-anhydro-2-deoxy-β-D-gulopyranose Hydrochloride[1]

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2-Amino-1,6-anhydro-2-deoxy-β-D-gulopyranose hydrochloride was synthesized from 2-acetamido-1,6-anhydro-2-deoxy-β-D-galactopyranose, and was found identical to the product isolated from streptothricin and streptolin B. It was further transformed into methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-gulopyranoside. An identical product was obtained from both synthetic and natural D-gulosamine hydrochloride.

In the hydrolyzates of streptothricin and streptolin B, van Tamelen and associates[3] isolated a parent substance of D-gulosamine (XV).[4] Paper chromatographic evidence showed that this substance was produced by the action of hydrochloric acid on XV. On the basis of its elementary analysis and its degradation by the periodate ion into *cis*-1,3-dioxolane-2,4-carboxaldehyde, the formula of 2-amino-1,6-anhydro-2-deoxy-β-D-gulopyranose hydrochloride (XIV) was proposed for this substance.[3]

The synthesis of a compound possessing structure XIV was started as an alternate route to the preparation of D-gulosamine,[5] before the already described synthesis had been successfully completed. This second synthesis has the advantage of using a more readily available starting material, D-galactose or D-lactose. Another reason for undertaking it, was to study the limits of the type of Walden inversion introduced in the carbohydrate field by Baker, *et al.*,[7] and used for the synthesis of D-allosamine[8] and D-gulosamine.[5] This inversion is based on the solvolysis of a sulfonyl group *trans*-vicinal to an acetamido group.

The 1,6-anhydro derivatives of 2-acetamido-2-deoxy-D-galactose have been already prepared by James, *et al.*[9] A modification of their method allowed the preparation, from either D-galactose or D-lactose, of large amounts of 1,6:2,3-dianhydro-β-D-talopyranose, subsequently transformed into the recently described[10] 2-acetamido-1,6-anhydro-2-deoxy-β-D-galactopyranose (I). Consideration of the spatial structure of the molecule showed the hydroxyls at positions 3 and 4 to have, respectively, axial and equatorial conformations. Direct introduction of the methylsulfonyl group with one mole of reagent gave the 4-*O*-methylsulfonyl derivative VI, but in low yield, whereas no isomer at position 3 could be isolated. When, however, compound I was treated with one mole of benzoyl chloride in pyridine solution, the position 3 possessing an axial conformation reacted surprisingly faster than the equatorial position, and the 3-*O*-benzoyl II and 4-*O*-benzoyl III derivatives were isolated in the respective yields of 48 and 38%, besides 4% of the 3,4-dibenzoate IV. Use of benzoic anhydride as the reagent[11] did not change significantly the respective amounts of the products obtained. In order to identify the location of the benzoyl groups introduced, a monomethylsulfonyl derivative V was prepared from II. It was subsequently hydrolyzed into 2-acetamido-1,6-anhydro-2-deoxy-4-*O*-methylsulfonyl-β-D-galactopyranose (VI), identical with the product described above. Methylation of VI gave the 3-*O*-methyl ether VII different from 2-acetamido-1,6-anhydro-2-deoxy-4-*O*-methyl-3-*O*-methylsulfonyl-β-D-galactopyranose (X), which had been synthesized from the previously prepared 4-methyl ether XI.[10] The same sequence of reactions was carried out with the 4-*O*-benzoyl derivative III through the intermediate steps VIII and IX to give finally a 4-*O*-methyl ether X identical to the one prepared from XI.

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(2) Special Investigator of the Arthritis and Rheumatism Foundation.

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(4) In the van Tamelen, *et al.*, communication[3] and in our publication,[5] the isolation of D-gulosamine has been referred to as the first isolation from natural sources of a sugar with the D-gulose configuration. Dr. T. Reichstein has kindly informed us of the previous isolation of 6-deoxy-D-gulose from α-antiarin.[6]

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