126. M.V.Likhosherstov and A.A.Petrov, Zhur.Obshch.Khim., 9, 2012 (1939).
127. W.Probst, Diss.Abs., 16, 657 (1956).
128. Yu.A.Baskakov and N.N.Mel'nikov, Zhur.Obshch.Khim., 29, 1233 (1959).
129. J.Conant and E.Jackson, J.Amer.Chem.Soc., 46, 1727 (1924).
130. E.Jackson, J.Amer.Chem.Soc., 48, 2166 (1926).
131. E.Jackson and J.Pasiut, J.Amer.Chem.Soc., 49, 2071 (1927).
132. E.Jackson, J.Amer.Chem.Soc., 56, 977 (1934).
133. M.V.Likhosherstov and S.V.Alekseev, Zhur.Obshch.Khim., 4, 1279 (1934).
134. M.V.Likhosherstov and R.A.Arkhangel'skaya, Zhur. Obshch.Khim., 7, 1914 (1937).
135. M.V.Likhosherstov, V.E.Zhabotinskaya, and L.D. Pavlovskaya, Zhur.Obshch.Khim., 8, 997 (1938).
136. M.V.Likhosherstov, R.A.Arkhangel'skaya, and T.V. Shalaeva, Zhur.Obshch.Khim., 9, 2085 (1939).
137. M.V.Likhosherstov and A.A.Petrov, Zhur.Obshch.Khim., 9, 2000 (1939).
138. R.E.Buckles, A.Langsjoen, R.E.Selk, and R.J.Coons, Proc.Iowa.Acad.Sci., 59, 170 (1952).
139. E.Schmidt *et al.*, Ber., 55, 2099 (1922); 57, 2039 (1924).
140. F.Böckemüller and W.Hoffmann, Annalen, 519, 164 (1955).
141. E.Schmidt *et al.*, Ber., 56, 1239 (1923); 59, 1279 (1926).
142. H.Meerwein, E.Bückner, and K.Emster, J.prakt.Chem., 152, 237 (1939).
143. W.A.Waters, "The Chemistry of Free Radicals" (Translated into Russian), Inostr.Lit., 1948, p.173.
144. A.V.Dombrovskii, Uspekhi Khim., 26, 689 (1957).
145. C.Coelsch and V.Boeckelhlide, J.Amer.Chem.Soc., 66, 412 (1944).
146. H.Lumbroso, L.Gasco, and C.Malen, Bull., 823 (1951).
147. A.N.Shidlovskaya, Ya.K.Syrkin, S.S.Novikov, A.A. Fainzil'berg, V.V.Sevost'yanova, and V.I.Gulevskaya, Dokl.Akad.Nauk SSSR, 132, 1376 (1960).
148. C.Wilson and H.Wenzke, J.Amer.Chem.Soc., 56, 2025 (1934).

Zelinskii Institute of Organic Chemistry,
Academy of Sciences of the USSR

A NEW FIELD IN THE STEREOCHEMISTRY OF SUGARS: THE STUDY OF THEIR CONFORMATIONS

B.N.Stepanenko

CONTENTS

I. Introduction 681
II. The conformations of monosaccharides 682
A. The conformations of six-membered monosaccharide rings 682
B. The conformation of five-membered monosaccharide rings 685
C. The conformation of acyclic forms of monosaccharides 685
III. The conformations of oligosaccharides 686
IV. The conformations of polysaccharides 686
V. Some particular aspects of the study of the conformations of carbohydrates 687
VI. The concept of conformations as applied to biochemistry 688

I. INTRODUCTION

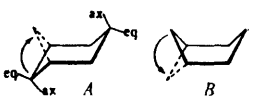
The last decade has been noteworthy for the introduction and vigorous development of a new field in the chemistry of carbohydrates — the study of their conformations[1-4]. It is no exaggeration to say that the new era in carbohydrate chemistry, this new development in the stereochemistry of sugars, is as significant as the period which followed the discovery of the cyclic forms of carbohydrates and the tautomerism between their open-chain and cyclic forms.

Despite this, data on the conformations of carbohydrates, which are relevant to chemists and biochemists, are little used in the USSR. The present knowledge of the conformations of carbohydrates is too broad to be encompassed within a single article, and therefore the object of this review is simply to set out the main theories and consider various examples which illustrate the practical value of this new field.

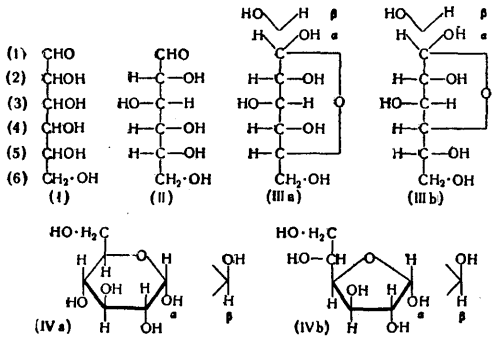
Conformations are the equilibrium forms of molecules which are taken up by aliphatic compounds because of the free rotation of groups around simple single bonds, or, in the case of cyclic compounds, because of rotation and change of the mutual positions of the individual parts of the molecule; in either case no bonds are broken in the mutual transitions between conformations, which distinguishes this from isomerisation.

In the case of cyclohexane, which may be considered as a model for a pyranose (the most common form of sugars), in addition to unstable transitional forms, the amount of which is usually negligible, two stable forms can exist: the chair form and the boat (or tub) form (Scheme 1). Of these the chair form is the more stable, since it contains the smaller number of mutually "screening" hydrogen atoms; it has 5.6 kcal less energy than the boat form, and under normal conditions it is the conformation taken up almost exclusively (99%) by cyclohexane.

The transition of one of these forms into the other can be considered as a rotation of part of the molecule, as shown by the arrows in Scheme 1, and is associated with a loss or gain of energy. Using the terms introduced into the conformational chemistry of sugars from the chemistry of



Scheme 1. The most stable conformations: *A* chair form, *B* boat form; ax = axial atoms or groups; eq = equatorial atoms or groups.



Scheme 2. Evolution of ideas on the structure of glucose (1870–1925).

alicyclic compounds, in the chair form of cyclohexane some of the hydrogen atoms are in positions almost in the plane of the molecule: these are called equatorial atoms. The other hydrogen atoms are in positions almost perpendicular to the plane of the molecule: these are called axial positions (Scheme 1).

Having considered the conversions of the conformational forms in the simplest case, cyclohexane, we shall now consider carbohydrates.

The evolution of our conceptions of the structure of monosaccharides (hexoses) is illustrated in Scheme 2.

After formula (I), suggested by Baeyer and Fittig (1870–1871), which only represents the presence of five alcohol groups and one aldehyde group in the molecule and is based mainly on Fischer's work (1890–1891), the configurational formula (II) was proposed (see my historical review[5]). Colley (1870) and Tollens (1883) first suggested cyclic formulae, which were then adapted into the formulae for α- and β-glucopyranose (IIIa) and glucofuranose (IIIb). In 1925 Haworth introduced the cyclic formulae which were the first attempt to reflect the geometry of the molecule as a six-membered ring, IVa and IVb. Such formulae have now been used for 25 years, but during the nineteen fifties they were replaced by conformational formulae which reproduce the form of the molecule more accurately and rationalise many facts which could not be previously explained.

We shall consider below the conformations of pyranoses, furanoses, and open-chain forms, and also oligo- and poly--saccharides.

II. THE CONFORMATIONS OF MONOSACCHARIDES

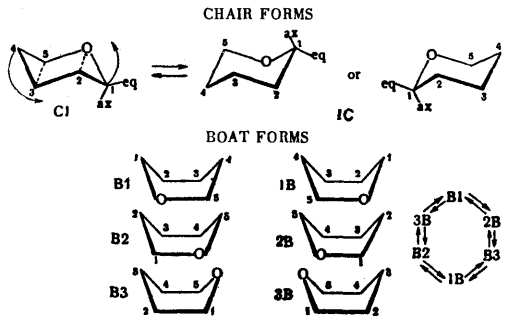
A. The Conformations of Six-Membered Monosaccharide Rings

Haworth's formulae are not completely satisfactory because a planar six-membered ring would be very strained and would not exist under normal conditions. Reeves[6,7] and others have shown that like cyclohexane, the pyranoses exist mainly in two forms which are less strained, the chair form and the boat form. These are very similar to the conformations of cyclohexanes, but with slightly less distortion in the ring and greater lability.

The cyclohexanes have two relatively stable conformations, but the pyranoses, because of the heteroatom and the asymmetric carbon atoms, have eight such conformations; two chair and six boat forms (Scheme 3). These are designated C, from the English word "chair", and B, from the English "boat", respectively. Three of the boat conformations (1, 2, and 3), as Scheme 3 shows, have the oxygen atom in different ring positions. In Scheme 3, the left-hand conformations are the mirror images of the right--hand series; this is indicated by the nomenclature C1 and 1C, B1 and 1B, etc. Although other systems exist[8,9], we shall use the nomenclature suggested by Reeves†.

The mutual conversions of the pyranose ring conformations may be represented as in the following example (Scheme 3): starting with the C1 form, rotation of the part of the molecule containing C_1 upwards and of the part containing C_4 downwards gives the 1C conformation (if this is imagined as turned through 180° it is seen that this conformation is the mirror image of the C1 conformation). It is very important to note that in such a conversion groups which are equatorial in the C1 conformation become axial in the 1C conformation, and *vice versa*. Rotation of the part of the molecule in the C1 conformation containing C_1 upwards but keeping all the other portions of the molecule as before gives the B1 conformation, and so on. The mutual conversions of the boat conformations are shown in the lower right in Scheme 3. For each pyranose, including α- and β-, there is the possibility of the indicated eight relatively stable conformations, without considering any transitional distorted forms.

In practice, however, the picture is somewhat simpler, since the various forms differ in stability. In the various



Scheme 3. Conformations of the pyranose ring. Below right: the possible conversions of conformation.

† It has been proposed[10] to indicate the positions of the hydroxyl groups, particularly the hemiacetal hydroxyl groups, by adding to the symbol for the basic chair or boat conformation (C, B1, B_2, B_3) the letter *A* or *E*, indicating the axial or equatorial position of the hemiacetal hydroxyl. The conformation is described in more detail by indicating the axial or equatorial position of each hydroxyl group, starting from the hemiacetal hydroxyl and then dealing with the others following the numeration of the carbon atoms. Thus the normal conformation of α-D-galactose is given by C(*aeeae*), the symbols *a* and *e* indicating the axial and equatorial positions of the hydroxyl groups starting from the first[11].

conformational conversions large groups normally tend to occupy equatorial positions; this effect, already known in the chemistry of alicyclic compounds from the work of Hassel and coworkers[12], is particularly important in the conformational analysis of sugars.

Reeves, one of the founders of this new chapter in the stereochemistry of sugars, has established[7] three main factors contributing to the instability of pyranose ring conformations:

(1) the presence of axial groups other than hydrogen atoms (for free sugars, the presence of axial hydroxyl groups);

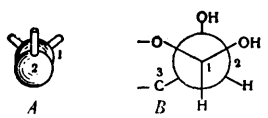
(2) the presence of the so-called Δ2 effect, *i.e.* the presence of an axial hydroxyl group at C_2, the C–O bond of which bisects the angle made by the two C–O bonds at C_1. The spatial distribution of the C–O bonds at C_1 and C_2 is shown in Scheme 4. This distribution, which produces an unfavourable dipole reaction in this conformation, is particularly effective in causing instability;

(3) the presence of an axial 5-hydroxymethyl group on the same side of the ring as other large axial substituents (the Hassel–Ottar effect)[12,13]. Reeves considered that these instability factors are additive, and evaluated the first as one unit for each large axial group, the Δ2 effect as 2.5 units, and the Hassel–Ottar effect as 0.5 unit. By considering these instability factors for each of the eight more stable conformations of a series of sugars and glycosides, Reeves could predict[7] which conformations would be most stable and would therefore predominate, and which would be least stable and would be practically non-existent.

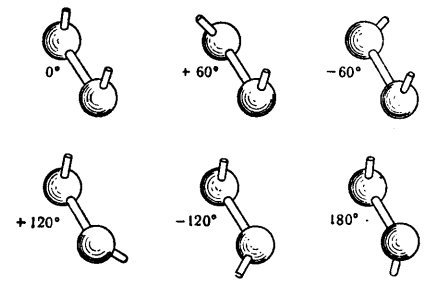
These predictions were confirmed with surprising accuracy in experiments made by Reeves himself and then by several others. We cannot refer to all the experimental data in detail, but shall mention some of them.

Reeves confirmed his predictions by studying the cuprammonium complexes of various substituted and unsubstituted glycosides. With dipolar groupings, *i.e.* two alcoholic hydroxyl groups, the cuprammonium ion forms a complex with a five-membered ring, containing two C atoms, two O atoms, and the cuprammonium ion. The following conditions are necessary for the formation of the complex: (*i*) the hydroxyl groups must be vicinal (with a few exceptions), and (*ii*) since neighbouring hydroxyl groups can be spatially directed on different sides, the distance between the centres of the oxygen atoms should not exceed a definite value, ~2.86 Å.

Scheme 5 shows the possible relative positions of bonds directed from neighbouring carbon atoms to the oxygen



Scheme 4. The Δ2 effect: the C–O bond at the second carbon atom bisects the angle formed by the C–O bonds on the first carbon atom. *A*) Model seen from the side of the second carbon atom; *B*) diagram seen from the side of the first carbon atom.



Scheme 5. The possible positions of bonds directed from neighbouring carbon atoms to the oxygen atoms of dipolar groups in all possible pyranose conformations.

atoms of the dipolar groupings in all possible conformations of pyranoses. If these directions are projected on to a plane perpendicular to the C–C bond, the angle between them will differ in each case, but will be practically constant in the stable conformations. By convention this angle is negative if it is measured in a clockwise direction as seen from the nearer group, and positive if measured in an anticlockwise direction. Complex-formation is possible only with projected angles of 0° and ±60°; only then is the distance between the two oxygen atoms less than the limiting value of 2.86 Å. With angles of ±120° and 180° the distance between the oxygen atoms is 3.45 and 3.71 Å respectively, and complex-formation does not take place.

Complex-formation can be detected most simply by spectrophotometry, or by the change in the plane of the optical rotation; the latter is of particular interest. There can be no change in the rotation, even in the presence of the complexing agent, if the projected angle between the C–O bonds of neighbouring atoms is 0°, since the resulting five-membered ring will be symmetrical. If the projected angle is ±60°, the five-membered ring in the resulting complex will be twisted and asymmetric and, depending on whether the projected angle is +60° or -60°, the resulting rotation will be dextro or laevo. For example, in glycopyranosides the 2-, 3-, and 4-hydroxyl groups are free, and Reeves selected substituted glycosides such that only 2,3-complexes or 3,4-complexes could be formed, and showed that the former, *i.e.* the 2,3-complexes, are laevorotatory, and the latter dextrorotatory. Thus by determining whether or not complex-formation takes place with a change in the rotation of the cuprammonium complexes or not, in various pyranosides and their substitution products, it was possible to evaluate the actual angles made by projection of neighbouring atoms in different parts of the molecule, and hence to find which conformation or conformations are typical for the given glycosides or their derivatives.

The Table gives very abbreviated data on the conformations found in the study of cuprammonium complexes, and the data predicted by evaluation of the instability factors. The correspondence is seen to be very good, except for the gulopyranosides, which exist in fact in only one conformation.

Many methods now exist for the determination of conformations. In addition to the study of cuprammonium complexes and the influence of alkalis on the rotation of sugars[14], other methods also find wide application. Chemical methods

TABLE.

Methylglycopyranoside	Type of complex	Conformation based on	
		complex-formation	calculation of instability factor
α-D-mannopyranoside	dextrorotatory	C1	C1
β-D-mannopyranoside	dextrorotatory	C1	C1
α-L-rhamnopyranoside	laevorotatory	1C	1C
α-D-altropyranoside	dextro-laevo--rotatory	C1, 1C	C1, 1C
β-D-altropyranoside	dextro-laevo--rotatory	C1, 1C	C1, 1C
α-D-gulopyranoside	dextrorotatory	1C	C1, 1C
α-D-idopyranoside	compensation	1C	1C
β-D-xylopyranoside	compensation	C1	C1
β-D-ribopyranoside	compensation	C1	C1
α-D-lyxopyranoside	dextro-laevo--rotatory	C1, 1C	C1, 1C

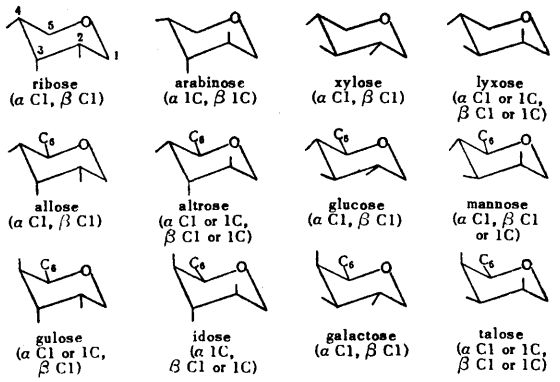
include the oxidation of free sugars with bromine by the Isbell–Pigman method[15]: an equatorial glycoside hydroxyl is much more readily oxidised. Physical methods include infra-red spectroscopy[16,17], which can distinguish between an equatorial and axial hydrogen at C_1 in aldopyranoses: the equatorial C_1–H bond shows a peak at 844 cm^{-1}, and the axial C_1–H bond a peak at 891 cm^{-1}. X-Ray structural analysis is used for crystalline sugars, *e.g.* α-D-glucose[18], α-L-rhamnose[19], and β-D-arabinose[20].

In recent years Lemieux and coworkers[21,22] have used proton magnetic resonance for studying conformations. Other methods have also been used, for instance the study of refraction (*e.g.* of methylglycosides[23]). In almost all cases these methods have confirmed Reeves' results obtained with the cuprammonium complexes.

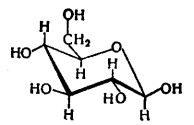
Correlating the results obtained with all the methods indicated, it can be said that the predominant conformation of α- and β-pyranoses and pyranosides is the chair form, usually C1; and of L-arabinosides, L-galactosides and certain others, the 1C form. Scheme 6 shows some of the more important penta- and hexa-pyranoses in the C1 conformation. With unstable C1 and 1C conformations of similar energies, both forms are present simultaneously (*e.g.* with lyxose, altrose, talose, and some others).

The most stable conformation is the C1 conformation of β-glucose (Scheme 7), which has no instability factors: all the hydroxyl groups and the hydroxymethyl group are equatorial. Glucose derivatives are also as a rule more stable than derivatives of other sugars[24]. Certain cases where α-glucose derivatives are most stable than β-glucose derivatives, which were inexplicable from the point of view of conformations (such as the greater stability of α-methyl-glucopyranoside[25], pentaacetyl-α-D-glucopyranose[26], and acetohalogeno-α-D-glucopyranose[27]), have found explanation from the electronic viewpoint[28].

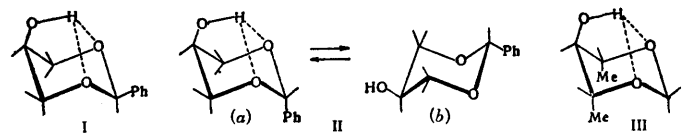
In speaking of the predominance of the chair form in glycosides and especially free pyranoses, it must not, of course, be forgotten that these forms are not infinitely stable; the possibility of their conversion into other (in particular, intermediate) forms cannot be excluded, especially under reaction conditions. Moreover, the solvent may exert a definite action. Water molecules in particular generally hinder the formation of intramolecular hydrogen bonds which might stabilise conformations which are sometimes rather unfavourable. In this connection we recall the model experiments of Stacey and coworkers[29] which showed that in dry CCl_4 *cis*-1,3-*O*-benzylideneglycerol is completely in the form in which there is solely hydrogen bonding with the hydrogen of the C_2 hydroxyl group of the glycerol (Scheme 8, I), while with the *trans*-form an equilibrium mixture exists (Scheme 8, II). In the former, the equatorial phenyl group favours hydrogen bonding. In the case of 1,5-didesoxy-2,4-*O*-methyleneribitol only the unfavourable conformation with two axial methyl groups shows hydrogen bonding (Scheme 8, III).



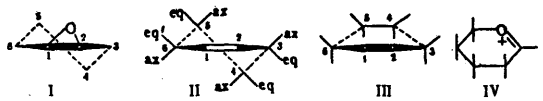
Scheme 6. The C1 conformations of some important pyranoses (pentoses and hexoses). These schematic conformational formulae show only the rings and the bonds leading to the hydroxyl groups and (in the case of the hexoses) the sixth carbon atom.



Scheme 7. The normal C1 conformation of glucose and glucosides.



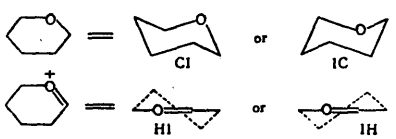
Scheme 8. The role of hydrogen bonding in the formation of conformations: I) *cis*-1,3-*O*-benzylideneglycerol; II) *trans*-1,3-*O*-benzylideneglycerol in two equilibrium conformations with *a*) axial and *b*) equatorial phenyl; III) 1,5-didesoxy-2,4-*O*-methyleneribitol.



Scheme 9. Models of intermediate conformations: I) cyclohexane oxide in the half-chair form; II) cyclohexene, in the half-chair form; III) the half-boat form; IV) the unsaturated oxonium ion.



Scheme 11. The furanose ring of fructo- and ribo-furanose. The atom C is 0.5 Å outside the plane of the remainder of the ring.



Scheme 10. The pyranose ring in the chair forms C1 and 1C, and the unsaturated oxonium ion produced from a pyranose, in the half-chair forms H1 and 1H.

In 1957 Reeves and coworkers directed attention to the significance of boat forms[14]. Although the chair form possesses the lower energy, in certain circumstances the energy difference may be very small, when the boat conformation allows all large substituents to be equatorial. The boat forms produced may be distorted and not geometrically exact. Such conformations can arise in the action of alkalis on glycosides; in particular they must be formed in polysaccharides (see below)

Half-chair forms. Intermediate forms, mainly the half-chair form, have recently begun to attract great attention. Alicyclic models of such forms are cyclohexane oxide [1,2-epoxycyclohexane (Ed. of Translation)][30] and cyclohexene[31]. Electron diffraction has shown that the 1, 2, 3, and 6 atoms in these compounds lie in one plane, with the 4 and 5 atoms below and above this plane (Scheme 9, I and II). Molecules may also exist in the half-boat form (Scheme 9, III), but all data indicate that these conformations are less stable than the half-chair form.

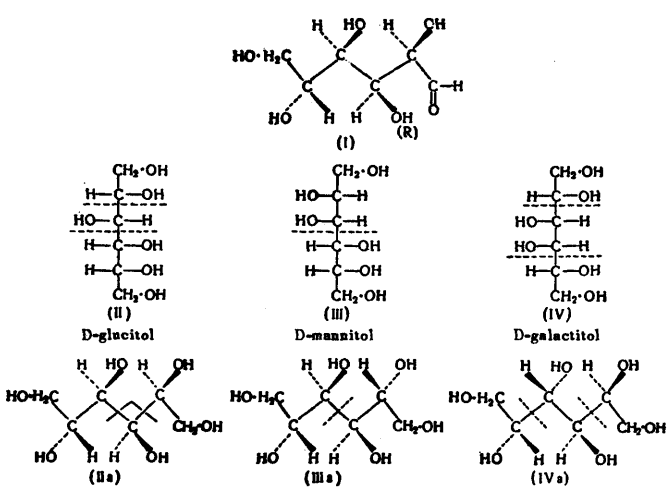
Half-chair conformations apparently arise in a number of chemical and biochemical reactions of sugars, and in particular the unsaturated sugar oxonium ion (Scheme 9, IV) which is formed in a number of reactions, apparently has this conformation, the reason being, as with cyclohexene, the presence of the double bond. This ion is formed as an intermediate in the hydrolysis of glycosides (see Section V.4.). The half-chair form is designated H; the mirror-image conformations are called 1H and H1 (Scheme 10).

B. The Conformation of Five-Membered Monosaccharide Rings

According to infra-red spectroscopy the rings of cyclopentane and tetrahydrofuran are not exactly planar, but the actual distortion is so small as to be negligible. Barker and Stephens have said[32] that the infra-red spectra of α- and β-furanosides are identical‡; the two anomers cannot be distinguished by this method, since both have the same angle of projection of the C_1–H bond on to the ring. The X-ray structural analysis of sucrose[33] and crystalline cytidine[34] showed that four of the furanose ring atoms in the fructofuranosyl and ribofuranosyl portions respectively are coplanar, while the fifth projects from this plane by 0.5 Å, and is in each case the C_4 atom, indicated by the letter C in Scheme 11. Apparently the slight bending of the molecule on the A–B axis facilitates the approach of substituents on atoms A, C, and B towards the plane of the ring. In solution the furanose ring may be quite appreciably folded, as is shown by periodate oxidation, which, irrespective of the configuration, causes rupture of the C_2–C_3 bond. Since oxidation by periodate ion proceeds by way of an intermediate complex with a diol[35], the bending for *trans*-diols must be considerable. It is interesting that periodate oxidation does not occur when bending is impossible because of fixation of the system by means of a bridge (*e.g.* in 1,6-anhydroglucofuranose).

C. The Conformation of Acyclic Forms of Monosaccharides

According to modern ideas, the carbon chain in acyclic forms of saccharides is a zig-zag, with the large substituents in zig-zag formation; thus glucose must have the conformation shown in Scheme 12 (I) which is quite unlike the Fischer formula. The following facts demonstrate this.



Scheme 12. The conformational formula of glucose (I), and the Fischer and conformational formulae of the hexitols glucitol (II) and (IIa), mannitol (III) and (IIIa), and galactitol (IV) and (IVa).

‡ The reader is advised to refer to the original on this point (Ed. of Translation).

In 1957 Schwarz[36], when oxidising hexitols with periodate, found that it is predominantly the *trans*-diol bonds which are ruptured, as shown by the dotted line in Scheme 12 (II) and (III); normally, however, periodate oxidises predominantly *cis*-diol systems. This unusual oxidation of hexitols is easily explained by considering the conformational formulae, which more nearly represent the actual structure. With gluticol, Scheme 12 (IIa), for example, it is seen that the hydroxyl groups at C_2, C_3, and C_4 are in fact in *cis*-positions, and they only appear to be *trans* because of the conventional manner of writing the Fischer formulae, which are projections of molecules with the carbon chain in the form of an arc with the convex side directed towards the observer.

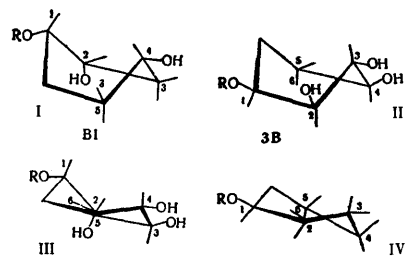
Even more convincing proof of the zig-zag conformation of acyclic forms was given by Hough and Bragg[37], who showed that large substituents R on C_3 hinder reactions by obstructing the approach of reagents to the 1-position, Scheme 12 (I), because of the zig-zag form of the molecule. Finally, Littleton[38] found an almost planar zig-zag carbon chain by X-ray structural analysis of a crystalline gluconate.

III. THE CONFORMATIONS OF OLIGOSACCHARIDES

The conformations of oligosaccharides have been comparatively little studied. As an example of such studies we adduce Bentley's work[39,40] on the conformations of cellobiose and maltose.

In cellobiose both residues have the C1 conformation (Scheme 13). The reducing residue is seen to have this conformation because of its rapid oxidation by bromine water (as in the oxidation of glucose), demonstrating the equatorial position of the hemiacetal hydroxyl. The requirement that both glucose residues should be equatorial has caused certain other suggested possible conformations for the non-reducing residue to be rejected. The X-ray structural study of crystalline cellobiose had already shown that both glucose residues are in the C1 conformation[41]. The same conformations have been found in cellulose[42].

The reducing glucose residue of maltose, like that of cellobiose, has the C1 conformation, as is demonstrated particularly by its rapid oxidation with bromine. With the α-1,4-glycoside linkage the non-reducing residue cannot have the C1 conformation, since the α-hemiacetal hydroxyl is axial, while the hydroxyl group at C_4 is equatorial. Results obtained in the study of the rate of hydrolysis of maltose, the rate of the periodate oxidation of α-methylmaltoside in comparison with the oxidation of certain other glycosides, the behaviour of cuprammonium complexes



Scheme 14. Possible conformations of the non-reducing residue in maltose (III and IV); they are intermediate between the boat conformations B1 and 3B (I and II).

and the action of alkalis, have shown[39,40] that the non-reducing glucose residue in maltose has a distorted conformation intermediate between B1 and 3B; all the properties of maltose confirm this conformation (Scheme 14).

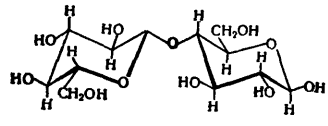
Cellobiose and maltose thus differ not only in having different glycosidic linkages (α and β), but also in the conformation of the non-reducing glucose residue.

IV. THE CONFORMATIONS OF POLYSACCHARIDES

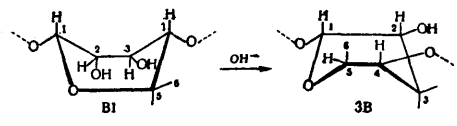
This field is the most complex and the least studied. In discussing the conformations of polysaccharides, two matters arise: (1) the conformation of the monosaccharide residues in the molecule, and (2) the shape of the whole molecule, which depends on the relative orientations of the monosaccharide residues, which can rotate around the glycoside linkages joining them.

The C1 conformation of the glucose residues in cellulose has been demonstrated[42].

The C1 conformation of the glucose residues of starch amylose is improbable, because the α-hemiacetal hydroxyl is axial and the OH at C_4 is equatorial. From study of the cuprammonium complexes and the action of alkalis, Reeves[43] concluded that the glucose residues of amylose exist not in one, but in two conformations, the boat forms B1 and 3B. Alkalis (which are known to change the properties of amylose) change the B1 conformation into the 3B conformation, *i.e.* all the glucose residues in "alkali amylose" are in the 3B conformation (Scheme 15). This change occurs because when the axial hydroxyl groups on the ring dissociate in alkaline medium they take up equatorial positions. These views may be open to some alteration in the light of Bentley's work, referred to above, on



Scheme 13. The conformational formula of cellobiose. Both glucose residues are in the C1 conformation.



Scheme 15. The B1 and 3B conformations of glucose residues in amylose. Alkali converts the B1 conformation into the 3B conformation.

the conformations of the hexose residues in maltose[40], but it is perfectly clear that there will still remain the hitherto unsuspected difference between cellulose and amylose, that the former contains the C1 conformation and the latter at least two conformations, boat forms or distorted forms close to boat forms.

The method of drying, the action of alkalis, and similar factors often greatly affect the properties of polysaccharides. The reason for these changes is probably to be sought in changes of the conformations of the monosaccharide residues, and much work remains to be done in the discovery of the actual causes and their mechanisms.

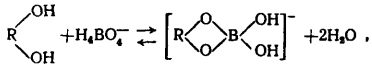
As regards the conformations of polysaccharide molecules as a whole, Everett and Foster recently[44] studied the conformations of amylose in solution, and from the viscosity of a number of amylose sub-fractions in various solvents (KCl and KOH solutions) they concluded that the molecules of these polysaccharides are bent chains but not spirals; the latter, which are more stable structures than rods, exist in a number of amylose complexes (with iodine, alcohols, etc.).

V. SOME PARTICULAR ASPECTS OF THE STUDY OF THE CONFORMATIONS OF CARBOHYDRATES

There are probably no reactions in carbohydrate chemistry for which conformational ideas are without any value, and a very large number of examples could be given where their value is particularly great. We shall refer to some of these.

1. Mutarotation. A good explanation has been given for the conversion of one tautomeric form of monosaccharide into several others, with one predominating, *e.g.* β-D-glucose in a glucose solution: the β-form of glucose has no axial hydroxyl groups while the hemiacetal hydroxyl of the α-form is axial, and this determines the predominance of the β-form. The anomers which are conformationally most stable predominate in the solutions of all sugars.

2. Reaction with borate and electrophoresis. The borate anion forms a complex with a diol of appropriate construction[45,46]:

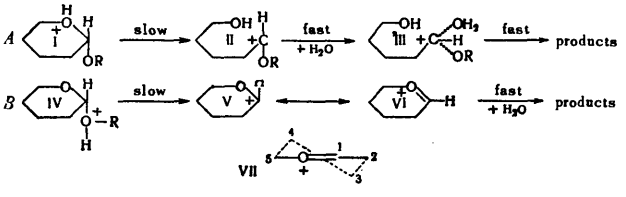


and because of the charge taken up by the sugar the complex will migrate on electrophoresis. The rate of migration of sugar depends on the position of equilibrium, and this depends on the stereochemistry of the dieol. Favourably disposed hydroxyl groups form stable complexes which have a high rate of migration. By studying the rates of migration of various substituted glucoses, Foster showed[47] that at pH 10 glucose can form three types of compound with borate: in the 2,4- and 4,6-positions of the open-chain form of glucose. Scheme 12 (I), and the 1,2-position (*cis*-diol) of the α-form of glucopyranose and glucofuranose. Other points of reaction are possible (*e.g.* 2,3-) but these have no substantial significance. Methylxylopyranoside does not migrate in borate solution, while methylarabinopyranoside migrates rapidly, because of the formation of a complex at the 3,4-diol position. These and a number of other facts[48] are in good agreement with conformational ideas, which can explain and to some extent predict the behaviour of various carbohydrates on electrophoresis.

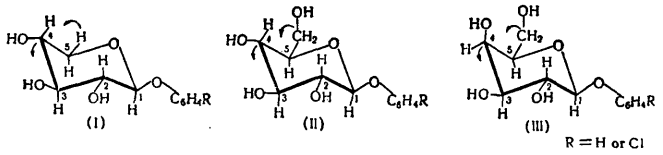
3. Chromatographic behaviour. There are indications[49] that an equatorial hydroxyl group in sugars is more strongly hydrated. It would be expected that a sugar with a small number of axial hydroxyl groups would be more soluble in water and would move more slowly in the corresponding systems. The R_f values of most free sugars can be calculated[2] from the formula $R_f = K + a(n + \alpha)$, where K and a are constants for a given series (*e.g.* the hexoses), n is the number of axial hydroxyl groups in the molecule, and α is the fraction of the anomeric sugar which in the given equilibrium solution contains the first hydroxyl group in the axial position (*e.g.* for glucose α-glucose has the hemiacetal hydroxyl in the axial position, and in solution it makes up ~0.34 of the total amount of sugar). In the above equation n and α depend on the conformation and thus the chromatographic mobility is directly dependent on conformation. A few sugars, such as arabinose and galactose, are exceptions, giving lower R_f values than those calculated by the formula.

4. Acid hydrolysis of glycosides. An enormous amount of factual material exists on the dependence of the rate of acid hydrolysis of glycosides on their structure. Two possible mechanisms for the hydrolysis have been discussed in the literature, one proposed by Bunton and coworkers[50] and Shafizade[51], and the other by Edwards[52].

According to the Bunton–Shafizade mechanism (Scheme 16, *A*) a proton adds to the pyranoside ring oxygen I, followed by a slow (and therefore rate determining for the overall hydrolysis) rupture of the pyranose ring and two further steps to the reaction products. According to the Edwards mechanism (Scheme 16, *B*), developed mainly from work on alkylglycosides, a proton attaches to the oxygen of the substituted hemiacetal hydroxyl IV, followed by the slow scission of an alcohol to form the carbonium or unsaturated oxonium ion VI; this reacts rapidly with water to give the final hydrolysis products. The unsaturated oxonium ion exists in the H1 half-chair conformation. The conversion of the C1 conformation in which most pyranosides exist into the H1 (Scheme 16, VII) is accompanied by a slight rotation of the atomic groups around the 2,3- and 4,5-bonds. Since the rate of hydrolysis is determined by the slow rate of formation of the H1 form, factors which influence the rotation of the atomic groups around the 2,3- and 4,5-bonds have a great significance; as always, large substituents tend to occupy equatorial positions. Edwards' mechanism explains a large amount of experimental material from a



Scheme 16. Mechanisms for the hydrolysis of glycosides: *A*) according to Bunton and Shafizade; *B*) according to Edwards.



Scheme 17. Xylosides (I), glucosides (II), and galactosides (III) in the C1 conformation.

single viewpoint, and is better than Bunton's in this respect[2]. In particular, it explains why alkylheptosides are more stable than alkylhexosides which, in their turn, are more stable than alkylpentosides[53].

Recent studies[54,55] on the kinetics of hydrolysis of phenyl- and chlorophenyl-glycosides have given results in good agreement with Edwards' views on conformations, and are in their turn additional facts which support his mechanism. Phenyl- and *p*-chlorophenyl-glucose, Scheme 17 (II), are hydrolysed less rapidly than the corresponding xylosides, Scheme 17 (I), because of the presence at C_5 of the large substituent CH_2OH, which on the formation of the half-chair form must leave the equatorial position and tend towards the axial position (Scheme 17). On the other hand, the hydroxyl group at C_4, which is axial in the galactosides , Scheme 17 (III), must move to a position close to equatorial when the half-chair form is produced, and this facilitates hydrolysis of the galactosides in comparison with the glucosides.

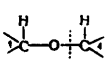
The fact that *p*-chlorophenylglycosides are hydrolysed less rapidly than the corresponding phenylglycosides may be explained by the lower electron density in the benzene ring of the aglycone chlorophenol, and suggests that the proton attaches to the oxygen of the substituted hemiacetal hydroxyl, rather than to the oxygen of the pyranoside ring.

VI. THE CONCEPT OF CONFORMATIONS AS APPLIED TO BIOCHEMISTRY

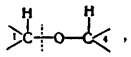
Conformational ideas have been little used in biochemistry.

We shall consider in some detail the work of Meyer and Larner[56] on the mechanism of action of α- and β-amylase. The problem which has been in existence for several decades is why polysaccharides of the starch type with 1,4--glucoside linkages are hydrolysed by α-amylase to α-maltose or its polymers, but by β-amylase to β-maltose.

Meyer and Larner first assumed that the different actions of the amylases are due to different points of attack. If the action of α-amylase is to break the glycoside bridge between the oxygen and C_4,

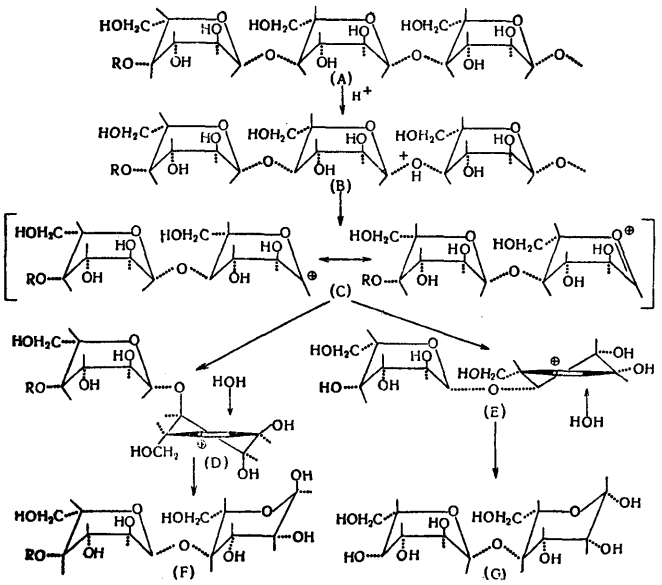


the configuration at C_1 should not change and the product would have an α-hemiacetal hydroxyl. If β-amylase breaks the bond between C_1 and the oxygen,



the reaction is taking place at the centre of asymmetry of C_1, and the configuration may change to give a β-glycoside hydroxyl. To decide this question, glycogen was hydrolysed with α- and β-amylase in $H_2{}^{18}O$; both enzymatically produced sugars were found to contain ^{18}O in the 1-position but not the 4-position. In a control experiment the maltose contained only traces of ^{18}O. Consequently, both α- and β-amylase break the glycoside bond at the same point, between C_1 and the oxygen. To explain the formation of both α- and β-hemiacetal hydroxyls, Meyer and Larner used Edwards' mechanism for the hydrolysis of glycosides (referred to above), with the intermediate formation of unsaturated oxonium ions in the half-chair conformation. Apparently the hydrolysis by α- and β-amylase proceeds as follows (Scheme 18).

The polyglycoside chain of the glycogen with the glucose residues in the B1 boat conformation (A) lies on the surface of the enzyme. A proton attaches to the oxygen bridge to form the saturated oxonium ion (B). Then the chain splits; the right-hand portion of molecule (B) with the new hydroxyl group undergoes no further change (until further attack by the enzyme) and is not shown. The left-hand portion,



Scheme 18. The mechanism of hydrolysis of glycogen at α- and β-amylase.

i.e. the portion split off (maltose in the case of β-amylase, when R = H, and maltose or its homologues in the case of α-amylase) is a carbonium or an oxonium ion (C). This unsaturated unstable ion takes on the more stable C1 conformation, and this occurs by way of a half-chair form with both α- and β-amylase, but because of the surface characteristics of the enzymes, with α-amylase the conversion proceeds through the H1 form (D), and with β-amylase through the 1H form (E). The final result is that the former gives an α- (F) and the latter a β-glucoside linkage (G).

The above mechanism of Meyer and Larner must be corrected in respect of the conformation of the non-reducing glucose residue in maltose (this should have a distorted boat conformation: see Section III). Although the mechanism cannot be considered fully proved, it gives the first satisfactory explanation of the different actions of α- and β-amylase based on modern ideas.

Under the influence of aldolases, the condensation of substances with a primary aldehydic group with aldehydes gives products having a dipolar group of threo-configuration, for example,

dihydroxyacetone phosphate + D-glyceraldehyde ⇌
⇌ D-fructose phosphate.

Hough and coworkers[57] have analysed this effect from the conformational aspect and shown its value in biosynthesis.

Conformational ideas have recently been used by Wallenfels[58] in considering the mechanism of action of galactosidase.

Conformational ideas should have particular value in the analyses of specific immunochemical reactions.

There can be no doubt that since the rate and sometimes even the possibility of chemical reactions depends on conformation this must apply at least equally to biochemical reactions. Clearly, conformations must be taken into account when interpreting the mechanisms of enzyme reactions.

1. R.Reeves, Ann.Rev.Biochem., 27, 15 (1958).
2. R.J.Ferrier and W.G.Overend, Quart.Rev., 13, 265 (1959).
3. G.Michel, Bull.Soc.chim.France, 2173 (1960).
4. B.N.Stepanenko, Uspekhi Khim., 28, 521 (1959).
5. B.N.Stepanenko, "'Aktivnye Formy' Prostykh Sakharov i Ikh Otnoshenie k Obmenu Uglevodov" (The "Active Forms" of Simple Sugars and Their Participation in Carbohydrate Exchange), Medgiz, 1945.
6. R.Reeves, J.Amer.Chem.Soc., 73, 957 (1951).
7. R.Reeves, Adv.Carbohydrate Chem., 6, 107 (1951).
8. J.Isbell, J.Res.Nat.Bur.Stand., 57, 171 (1956).
9. R.D.Guthrie, Chem.and Ind., 1593 (1958).
10. H.Isbell and R.Tipson, Science, 130, 793 (1959).
11. H.Isbell and R.Tipson, J.Res.Nat.Bur.Stand., 64A, 171 (1960).
12. O.Hassel, Quart.Rev., 7, 221 (1953).
13. O.Hassel and B.Ottar, Acta Chem.Scand., 1, 929 (1947).
14. R.Reeves and F.A.Blouin, J.Amer.Chem.Soc., 79, 2261 (1957).
15. H.S.Isbell and W.Pigman, J.Res.Nat.Bur.Stand., 10, 337 (1932).
16. W.Brock-Neely, Adv.Carbohydrate Chem., 12, 13 (1957).
17. H.S.Isbell, F.Smith, *et al.*, J.Res.Nat.Bur.Stand., 59, 41 (1957).
18. T.R.R.McDonald and C.A.Beevers, Acta Cryst., 5, 654 (1952).
19. H.McD.McGeachin and C.A.Beevers, Acta Cryst., 10, 227 (1957).
20. S.Furberg and A.Hordvik, Acta Chem.Scand., 11, 1594 (1957).
21. R.Lemieux, R.Kullnig, H.Bernstein, and W.Schneider, J.Amer.Chem.Soc., 80, 6098 (1958).
22. R.Lemieux and R.Kullnig, J.Amer.Chem.Soc., 79, 1005 (1957).
23. R.Kelly, Canad.J.Chem., 35, 149 (1957).
24. R.Hansen and E.Craine, J.Biol.Chem., 208, 293 (1954).
25. G.N.Bollenbeck, "Methyl Glucoside: Preparations, Physical Constants, Derivatives", Academic Press, New York, 1958, p.12.
26. W.Bonner, J.Amer.Chem.Soc., 73, 2659 (1951).
27. R.Lemieux, Adv.Carbohydrate Chem., 9, 1 (1954).
28. T.Edwards, Chem.and Ind., 1102 (1955).
29. J.Brimacombe, A.Foster, and M.Stacey, Chem.and Ind., 1228 (1958).
30. B.Ottar, Acta Chem.Scand., 1, 283 (1947).
31. D.Barton, R.Cookson, W.Klyne, and C.Shoppee, Chem. and Ind., 21 (1954).
32. G.Barker and Stephens, J.Chem.Soc., 4550 (1954).
33. C.A.Beevers and W.Cochran, Proc.Roy.Soc., 190A, 257 (1947).
34. S.Furberg, Acta Cryst., 3, 325 (1950).
35. G.Buist and C.Bunton, J.Chem.Soc., 1406 (1954); 4567, 4580 (1957).
36. J.C.Schwarz, J.Chem.Soc., 276 (1957).
37. L.Hough and P.Bragg, J.Chem.Soc., 4347 (1957).
38. C.D.Littleton, Acta Cryst., 6, 775 (1953).
39. R.Bentley, "Abstracts Division of Carbohydrate Chemistry", Amer.Chem.Soc., 132nd Meeting, New York, September, 1957, 6D.
40. R.Bentley, J.Amer.Chem.Soc., 81, 1952 (1959).
41. J.Wunderlich, Compt.rend., 240, 1350 (1955).
42. W.Astbury and M.Davies, Nature, 154, 84 (1944).
43. R.Reeves, J.Amer.Chem.Soc., 76, 4595 (1954).
44. W.Everett and J.Foster, J.Amer.Chem.Soc., 81, 3464 (1959).
45. A.Foster, Adv.Carbohydrate Chem., 12, 81 (1957).
46. J.Boeseken, Adv.Carbohydrate Chem., 4, 189 (1949).
47. A.Foster, J.Chem.Soc., 982 (1953).
48. A.Foster, J.Chem.Soc., 1395 (1957).
49. M.Kabagana and D.Patterson, Canad.J.Chem., 36, 563 (1958).
50. C.Bunton, T.Lewis, and D.Llewellyn, Canad.J.Chem., 4419 (1955).
51. F.Shafizade, Adv.Carbohydrate Chem., 13, 9 (1958).
52. T.Edwards, Chem.and Ind., 1102 (1955).
53. H.S.Isbell and H.L.Frush, J.Res.Nat.Bur.Stand., 24, 125 (1940).
54. B.N.Stepanenko and O.G.Serdyuk, "Uglevody i Uglevodnyi Obmen" (Carbohydrates and Carbohydrate Exchange), Izd. Akad.Nauk SSSR, 1962, p.40.
55. B.N.Stepanenko and O.G.Serdyuk, Dokl.Akad.Nauk SSSR, 139, 1132 (1961).
56. F.Meyer and J.Larner, J.Amer.Chem.Soc., 81, 188 (1959).
57. L.Hough and J.K.Jones, Adv.Carbohydrate Chem., 11, 185 (1956).
58. K.Wallenfels, Bull.Soc.Chim.biol., 42, 1715 (1960).

Bakh Institute of Biochemistry,
Academy of Sciences of the USSR