NEWER ASPECTS OF THE STEREOCHEMISTRY OF CARBOHYDRATES

By R. J. FERRIER, PH.D., and W. G. OVEREND, D.SC.
(BIRKBECK COLLEGE, UNIVERSITY OF LONDON)

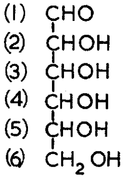
ALTHOUGH the term "conformation" was first used by W. N. Haworth in 1929[1] in connexion with sugars, the principles of conformational analysis were developed later, mainly in the realm of alicyclic chemistry. Nowadays interest is being focussed to an increasing extent on the detailed stereochemistry of carbohydrates, and the application of these principles has proved fruitful.

For discussions of stereochemistry it is important that the formulæ used to depict compounds should give as accurate a representation as possible of the molecular shape. The earliest formula (*e.g.*, I) for sugars gave only an indication of the functional groups present in the molecules. Later developments took into account classical stereochemical features, but only recently has serious consideration been given to the graphical representation of the detailed shapes of sugars.

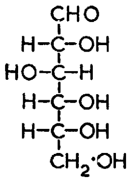
Historical aspects of the conventions adopted for the Fischer (straight-chain) formulæ for sugars (*e.g.*, II) have been adequately discussed by Hudson.[2] Such formulæ are still of value as the simplest representation of aldoses and ketoses, and probably cause the student least difficulty. Early in the development of carbohydrate chemistry it became apparent that sugars had properties which could only be reconciled by postulating cyclic structures of the hemiacetal type (*e.g.*, III); the existence of α- and β-forms was explicable owing to asymmetry at $C_{(1)}$. In the D-series the more dextrorotatory sugar of an anomeric pair was designated as the α-form,[3] whereas in the L-series such a compound was of the β-type. It is possible for the cyclic structures to be either five- or six-membered—the so-called furanose (*e.g.*, IIIb) and pyranose (*e.g.*, IIIa) sugars,[4] and Drew and Haworth[5] introduced the now well-known perspective formulæ (*e.g.*, IV) to give a more accurate representation of these cyclic forms. Formulæ (I—IV) show the progressive development in the representation of D-glucose. For many years, Haworth formulæ proved to be satisfactory and served for the development of descriptive carbohydrate chemistry as it is known today, but they are not so useful in discussions of mechanisms of some reactions and of the detailed stereochemistry of sugars. In the hope that a more thorough understanding of these problems would result, the ideas of conformational analysis (see ref. 6) have been

---

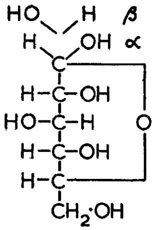
[1] Haworth, "The Constitution of the Sugars", E. Arnold and Co., London, 1929, p. 90.
[2] Hudson, *Adv. Carbohydrate Chem.*, 1948, **3**, 1.
[3] *Idem*, *J. Amer. Chem. Soc.*, 1909, **31**, 66.
[4] Goodyear and Haworth, *J.*, 1927, 3136.
[5] Drew and Haworth, *J.*, 1926, 2303.
[6] Barton and Cookson, *Quart. Rev.*, 1956, **10**, 44.



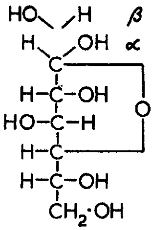
(I)



(II)

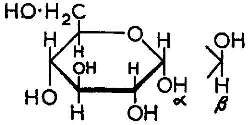


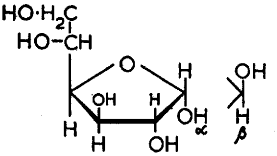
(IIIa)



(IIIb)

(IVa)





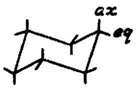
(IVb)

applied. Some consequences of this development will be discussed in this Review.

Six-membered Rings

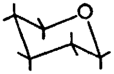
Six-membered (pyranose) ring systems will be considered in greatest detail because sugars and their derivatives exist and react most frequently in this form.

Extensive investigations with cyclohexane[7] have shown that it exists preferentially in the chair (V) rather than the boat form (VI). In this



(V)

(VI)



(VII)

preferred form the non-bonded interactions of the axially (*ax*) and equatorially (*eq*) disposed hydrogen atoms are minimised. It has been demonstrated by Hassel and his colleagues[7] that the preferred conformation of substituted cyclohexanes is that which has most of the substituents in equatorial positions. (The free-energy difference between the cyclohexanols with an axial or an equatorial hydroxyl group is about 0.8 kcal./mole.[8])

The substitution of an oxygen atom for carbon in the cyclohexane ring, leading to the basic skeleton (VII) of pyranose sugars, causes only minor distortions in the ring,[9] and so the general conformational features are retained. It might, however, be expected that the heterocyclic ring will be slightly more readily distorted than the cyclohexane ring owing to the

---

[7] Hassel, *Quart. Rev.*, 1953, 7, 221; see also ref. 6.
[8] Cookson, *Ann. Reports*, 1957, **54**, 170.
[9] Hassel and Ottar, *Acta Chem. Scand.*, 1947, **1**, 929.

elimination of interactions of two hydrogen atoms which are replaced by lone pairs of electrons. Unfortunately, we have to consider for the heterocyclic ring more strainless forms than for cyclohexane. Reeves[10] has designated the possible regular forms as in Fig. 1, but more recently he has pointed out that there is an infinite number of skewed conformations all devoid of angle strain.[11] Although it is convenient to discuss pyranose conformation in terms of regular geometrical structures, it is necessary to note that distortions are extremely likely, especially in the course of reactions. Other conventions have been proposed[12] but, for the purpose of this Review, Reeves's symbols are adopted. Formulæ (VIII) and (IX) will be used throughout to represent the pyranose ring in the C1 and 1C conformations, as they are most easily related to the usual Haworth formulæ.

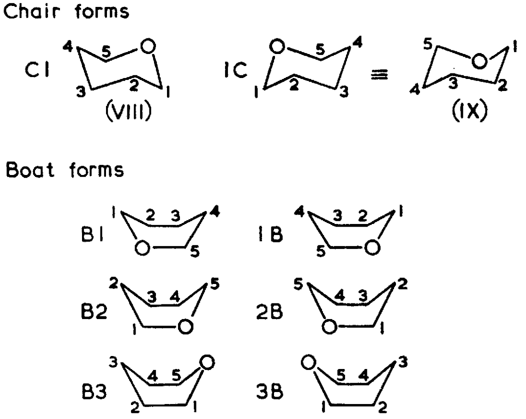


FIG. 1. *The eight regular strainless conformations of the pyranose ring with Reeves's symbols*

Reeves[10] concluded that whenever possible a chair form is preferred to a boat form. He assigned additive instability units of arbitrary value to the following features: (i) axial groups other than hydrogen atoms, (ii) an axial 2-hydroxyl group of which the C–O bond bisects the angle between the two $C_{(1)}$–O bonds ["△2 effect" caused probably by unfavourable dipolar interactions (cf. p. 268)], and (iii) an axial 5-hydroxymethyl group on the same side of the ring as another bulky axial substituent ("Hassel–Ottar effect"). Thus he was able to predict the favoured conformation of pyranose sugars of various configurations.

By detailed study of the complexes which cuprammonium forms with diol groups in the molecules, he showed the validity of his predictions. By using molecules containing a 1,2-diol system with the hydroxyl groups

---

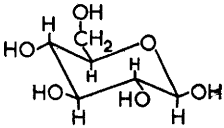
[10] Reeves, *Adv. Carbohydrate Chem.*, 1951, **6**, 107.

[11] *Idem*, *Ann. Rev. Biochem.*, 1958, **27**, 15.

[12] Isbell, *J. Res. Nat. Bur. Stand.*, 1956, **57**, 171; Guthrie, *Chem. and Ind.*, 1958, 1593.

held in fixed relations[10] it was found that the cuprammonium reagent formed complexes only when the hydroxyl groups had a projected angle of 0° (*i.e.*, were truly *cis*) or ± 60°. If the projected angle between the hydroxyl groups was greater than 60°, complex formation did not occur. By working with suitably substituted derivatives of methyl β-D-glucopyranoside Reeves[10] was able to demonstrate that a lævorotatory complex is formed at the 2,3-position, whereas with the 3,4-diol the complex is dextrorotatory. Little or no complex-formation occurs elsewhere in the molecule. The only possible conformations which would permit the formation of these complexes are C1 and 3B. Since the 4,6-*O*-ethylidene derivative, which cannot assume the 3B conformation, behaves like the 4-*O*-methyl ether, it is believed that all the glucosides react with cuprammonium in the C1 chair form. A range of methyl glycopyranosides was examined[10] by this procedure, and excellent correlation was noted between the conformations deduced from the complex-forming reactions and those predicted on the basis of the assigned instability units.

In the C1 conformation (X) β-D-glucopyranose has all the hydroxyl groups and the hydroxymethyl substituent in equatorial positions. According to Reeves's ideas it has no instability factors and so this would be expected to have the most stable ring. Glucose derivatives are found to be more stable than derivatives of other sugars. For example, the free-energy difference between α-D-glucose 1-phosphate and α-D-galactose 1-phosphate was found to be 0·7 kcal./mole.[13] Rather surprisingly, however, 1-substituted derivatives of α-D-glucose generally are more stable than the corresponding β-analogues, *e.g.*, equilibrium mixtures of methyl D-glucopyranosides,[14] penta-*O*-acetyl-D-glucopyranoses,[15] and acetohalogeno-D-glucopyranoses[16] all contain more of the α- than of the β-form. This apparent anomaly is explained by Edward[17] in terms of dipole–dipole interaction between the lone-pair electrons on the ring-oxygen atom and the polar group on $C_{(1)}$; this favours the α-form.



(X)

The representation of any sugar in the C1 conformation is probably best done by relating it to β-D-glucose in either the Fischer or the Haworth formula and making the necessary alterations to the all-equatorial model. The D-aldo-pentoses and -hexoses in the C1 conformation are shown in

---

[13] Hansen and Craine, *J. Biol. Chem.*, 1954, **208**, 293.

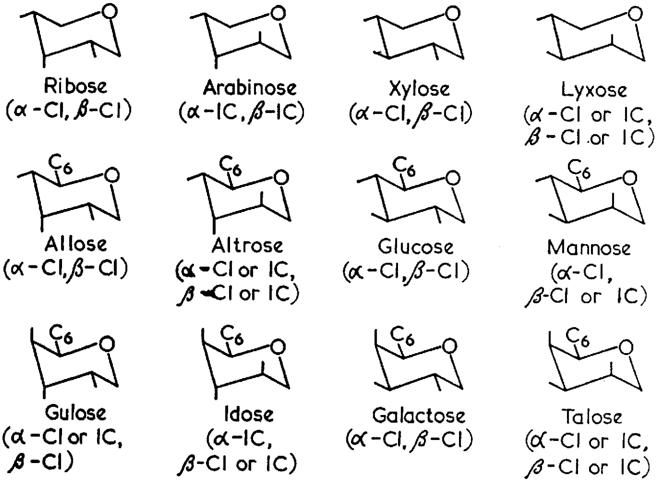
[14] Bollenbeck, "Methyl Glucoside", Academic Press Inc., New York, 1958, p. 12.

[15] Bonner, *J. Amer. Chem. Soc.*, 1951, **73**, 2659.

[16] Lemieux, *Adv. Carbohydrate Chem.*, 1954, **9**, 1; Haynes and Newth, *ibid.*, 1955, **10**, 207.

[17] Edward, *Chem. and Ind.*, 1955, 1102.

Fig. 2. The more stable chair forms (as determined by Reeves and indicated in parenthesis) are not rigidly fixed. Instances are known in which molecules react in other conformations, but in the sugar series little is known about the energy differences between conformations or their energies of interconversion. Angyal and McHugh,[18] working with inositols, recently calculated interaction energies of neighbouring groups in cyclohexane rings, but we find that application of their values to the pyranoses does not give results in good agreement with those of Reeves. Barker and Shaw[19] have approached the problem from a purely geometrical viewpoint, and after calculating total atomic overlaps for each chair form of the pyranoses, have predicted the preferred conformations. The results obtained in many cases agree with those of Reeves, but their approach is probably oversimplified in that it deals solely with steric factors in non-solvated molecules. Reeves's method on the other hand suffers from the drawback that no alteration in shape of the sugar ring is envisaged during complex-formation. It must be borne in mind that the non-formation of a complex is important in Reeves's derivation of conformation, and the above criticism does not apply in these circumstances.



Ribose (α-C1, β-C1)

Arabinose (α-1C, β-1C)

Xylose (α-C1, β-C1)

Lyxose (α-C1 or 1C, β-C1 or 1C)

Allose (α-C1, β-C1)

Altrose (α-C1 or 1C, β-C1 or 1C)

Glucose (α-C1, β-C1)

Mannose (α-C1, β-C1 or 1C)

Gulose (α-C1 or 1C, β-C1)

Idose (α-1C, β-C1 or 1C)

Galactose (α-C1, β-C1)

Talose (α-C1 or 1C, β-C1 or 1C)

FIG. 2. *The* D-*aldo-pentoses and -hexoses represented in the* C1 *conformation. The stable conformation of the anomers as predicted by Reeves*[10] *are indicated in parenthesis. (Substituents other than hydrogen are denoted at* $C_{(2)}$–$C_{(6)}$.)

Lately infrared spectroscopy[20] and proton magnetic resonance studies[21] have provided alternative experimental approaches to the assignment of stable conformations. By these methods equatorial and axial glycosidic bonds can be differentiated, and results indicate that the common sugars

---

[18] Angyal and McHugh, *Chem. and Ind.*, 1956, 1147.

[19] Barker and Shaw, *J.*, 1959, 584.

[20] Brock-Neely, *Adv. Carbohydrate Chem.*, 1957, **12**, 13.

[21] Lemieux, Kullnig, Bernstein, and Schneider, *J. Amer. Chem. Soc.*, 1958, **80**, 6098.

exist in the conformations predicted by Reeves. Moreover, Lemieux *et al.*[21] have demonstrated that the configurations assigned to the α- and the β-anomers of sugar acetates on the basis of Hudson's rules[22] are correct.

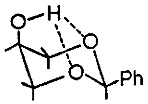
In 1957 Reeves and Blouin,[23] as a result of the study of the effect of alkali on the optical rotation of solutions of glycopyranosides, were led to reconsider the importance of boat forms. They concluded that, although chair forms are generally of lower energy, in certain circumstances the difference may be offset when a boat conformation allows all bulky substituents to be equatorial. Rather than geometrically symmetrical structures it is suggested that distorted boats occur in which the tendency for large groupings to become equatorial is balanced by non-bonded interactions brought about by groupings approaching eclipsed positions. Previously Reeves[24] had suggested, in agreement with Freudenberg and Cramer,[25] that amylose, the linear component of starch, contains α-D-glucopyranose units in the B1 or 3B conformation which allows the polymeric 1- and 4- substituents to assume equatorial positions. Greenwood and Rossotti[26] on the other hand have decided, on the evidence of infrared absorption studies on the amylose–iodine complex, that the pyranose units of amylose have chair (C1) rather than boat forms.

X-Ray crystallographic measurements have shown that α-D-glucose,[27] methyl β-D-xylopyranoside,[28] the pyranose ring in sucrose,[29] α-D-glucosamine hydrobromide,[30] α-L-rhamnose,[31] and β-D-arabinose[32] all exist in the crystalline form in a chair conformation and that this conformation is, in all cases, that predicted by Reeves. This cannot, however, be taken as proof that these compounds assume in solution exclusively the same chair conformations, as the nature of inter- and intra-molecular bonding forces differ in the solid state and in solution. Hydrogen-bonding would be expected to influence the fine structure of sugars, particularly in solution. There is good evidence for hydrogen-bonding to water molecules,[33] and recent work with simple model compounds has indicated that intramolecular hydrogen bonds may be significant in fixing conformation. Evidence which clearly demonstrates this has been given by Brimacombe, Foster, and Stacey[34] who showed spectroscopically that in dry carbon

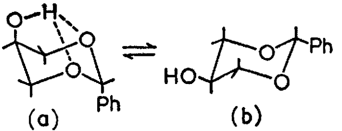
---

[22] Hudson, *J. Amer. Chem. Soc,*, 1916, **38**, 1566.
[23] Reeves and Blouin, *J. Amer. Chem. Soc.*, 1957, **79**, 2261.
[24] Reeves, *J. Amer. Chem. Soc.*, 1954, **76**, 4595.
[25] Freudenberg and Cramer, *Chem. Ber.*, 1950, **83**, 296.
[26] Greenwood and Rossotti, *J. Polymer Sci.*, 1958, **27**, 481.
[27] McDonald and Beevers, *Acta Cryst.*, 1952, **5**, 654.
[28] Brown, Ph.D. Thesis, Birmingham, 1939.
[29] Beevers and Cochran, *Proc. Roy. Soc.*, 1947, *A*, **190**, 257.
[30] Cox and Jeffrey, *Nature*, 1939, **143**, 894.
[31] McGeachin and Beevers, *Acta Cryst.*, 1957, **10**, 227.
[32] Furberg and Hordvik, *Acta Chem. Scand.*, 1957, **11**, 1594.
[33] Kabayana and Patterson, *Canad. J. Chem.*, 1958, **36**, 563, and refs. therein.
[34] Brimacombe, Foster, and Stacey, *Chem. and Ind.*, 1958, 1228; see also Brimacombe, Foster, Stacey, and Whiffen, *Tetrahedron*, 1958, **4**, 351.

tetrachloride solution *cis*-1,3-*O*-benzylideneglycerol (XI) is completely hydrogen-bonded, whereas the *trans*-isomer (XII) exists as an equilibrium mixture of the bonded (a) and the non-bonded (b) form. The appreciable amount of (XIIa) and the 20—30% of form (XIII) found in 1,5-dideoxy-2,4-*O*-methyleneribitol indicate that in carbon tetrachloride solution bonding of this type could be sufficiently strong to hold pyranoid rings in what otherwise would be very unfavourable conformations. It would be expected, however, that in aqueous solution the intermolecular bonding to solvent molecules would significantly diminish the effect of intramolecular hydrogen-bonding of this type.

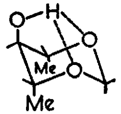


(XI)



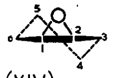
(a) (b)

(XII)

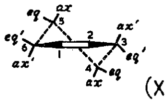


(XIII)

Half-chair Forms.—It has been shown by electron-diffraction that cyclohexene oxide[35] (XIV) exists, like cyclohexene[36] (XV), in the half-chair form, *i.e.*, $C_{(1)}$, $C_{(2)}$, $C_{(3)}$, and $C_{(6)}$ are coplanar and the other methylene groupings are in staggered positions. The 4- and 5-hydrogen atoms are in true axial and equatorial positions, but those at $C_{(3)}$ and $C_{(6)}$ become displaced and so are termed quasi-equatorial (*eq'*) and quasi-axial (*ax'*).[36] It is possible that these molecules could exist in half-boat forms (XVI), but physical and chemical evidence[36] together with thermodynamic data[37] suggest that the half-chair conformation is the more stable. Structures of similar shape occur in sugar derivatives which have a feature constraining four consecutive atoms of the pyranoid ring in a plane, *e.g.*, a double bond as in glycals, or an epoxide ring. Intermediates of the type (XVII) occur in some sugar reactions. Although these do not necessarily take up a half-chair, they probably are in the form of the half-chair oxonium ion (XVIII).

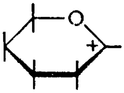


(XIV)

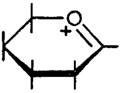


(XV)

(XVI)



(XVII)



(XVIII)

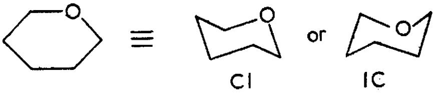
Half-chair conformations can be named by reference to the chair forms; thus a glycal derived from a sugar in a C1 conformation could, we propose, be considered to be in the H1 conformation. The alternative half-chair conformation is the 1H.

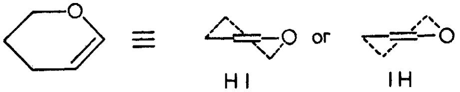
---

[35] Ottar, *Acta Chem. Scand.*, 1947, **1**, 283.

[36] Barton, Cookson, Klyne, and Shoppee, *Chem. and Ind.*, 1954, 21.

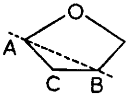
[37] Beckett, Freeman, and Pitzer, *J. Amer. Chem. Soc.*, 1948, **70**, 4227.





Five-membered Rings

From infrared studies[38] on cyclopentane and tetrahydrofuran it has been deduced that although the rings are not quite planar the puckering is so small that the molecules are essentially flat. An indication that furanoid rings are also flat was given by Barker and Stephens[38] who found that α- and β-furanosides could not be differentiated by infrared spectroscopy; both anomers had the same projected angle from the ring for the $C_{(1)}$–H bond. In the fructofuranosyl portion of crystalline sucrose[29] and in the ribofuranosyl part of crystalline cytidine[39] four of the ring atoms are coplanar and the fifth is 0·5 Å out of this plane. In both cases the atom which is displaced occupies the same relative position in the furanosyl ring, *i.e.*, 4 in the ketofuranoside and 3 in the aldofuranoside. It is considered that a rotation about the axis shown dotted in (XIX) facilitates lattice packing by bringing the larger substituents at positions A and B nearer to the plane of the ring, and the hydroxyl at C almost exactly into that plane. There is evidence that in solution the ring is flexible. Thus, irrespective of configuration, furanosides are oxidised at $C_{(2)}$–$C_{(3)}$ by the periodate ion *via* a cyclic intermediate, the formation of which must cause considerable distortions particularly when the diol is *trans*. Such distortions are impossible when the ring is locked by a bridge system (cf. 1,6-anhydroglucofuranose, which is not oxidised).



(XIX)

Relative Stability of Pyranoid and Furanoid Rings.—By analogy with results obtained in other branches of aliphatic chemistry[40] (see Table 1) it would be expected that free sugars would exist preferentially in the six-membered pyranose ring form. On the other hand, a five-membered ring would be predicted for lactones derived from sugar acids. These expectations are in accord with the well-established facts of carbohydrate

---

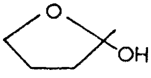
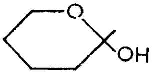
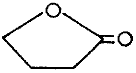
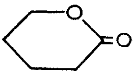
[38] Barker and Stephens, *J.*, 1954, 4550, and refs. therein.

[39] Furberg, *Acta Cryst.*, 1950, **3**, 325.

[40] Brown, Brewster, and Shechter, *J. Amer. Chem. Soc.*, 1954, **76**, 467.

chemistry. That glycosides also are more stable in the six-membered ring form is illustrated by the fact that an equilibrium mixture of pyranosides and furanosides contains only small amounts of the latter.[41] Brown, Fletcher, and Johannsen[42] have discussed the reactivity of groupings attached to five- and six-membered rings and have attributed the relative stability of the cyclohexane and the cyclopentanone derivatives to internal ring strains which arise during the formation of reaction intermediates.

TABLE 1.

Compound	Derived cyclic structure	% of cyclic form at equilibrium
γ-Hydroxy-butyraldehyde		88.6
δ-Hydroxy-valeraldehyde		93.9*
γ-Hydroxy-butyric acid lactone		72.8
δ-Hydroxy-valeric acid lactone		9.0

(*Sugars have much higher proportions of cyclic structures. This conforms with the generalisation that substitution favours ring-formation.[41])

Acyclic Sugars

A carbon chain takes up a zig-zag conformation with large substituents in staggered positions.[7,43] Consequently the acyclic form of D-glucose would be expected to exist as (XX; R=H), a representation which obviates the misconceptions which can arise with the Fischer formula. Working with hexitols Schwarz[44] has shown that preferential attack by periodate occurs at diol systems which are represented as *trans* (*threo*-configuration) in the Fischer projection formulæ, see (XXI)—(XXIII). Periodate normally oxidises preferentially a *cis*-α-glycol (*erythro*-configuration), but the apparent anomaly is readily explicable by reference to formulæ (XXIa)—(XXIIIa).

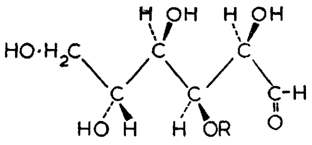
---

[41] Hammond in "Steric Effects in Organic Chemistry", ed. Newman, Wiley and Sons, Inc., New York, 1956, p. 425.

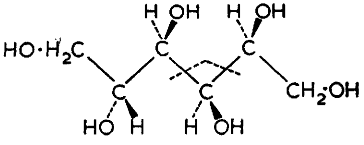
[42] Brown, Fletcher, and Johannsen, *J. Amer. Chem. Soc.*, 1951, **73**, 212.

[43] McCoubrey and Ubbelohde, *Quart. Rev.*, 1951, **5**, 364.

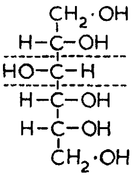
[44] Schwarz, *J.*, 1957, 276.



(XX)

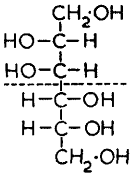


(XXIa)



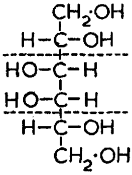
D-Glucitol

(XXI)



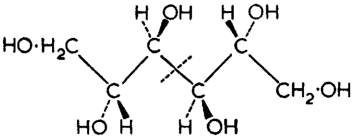
Mannitol

(XXII)

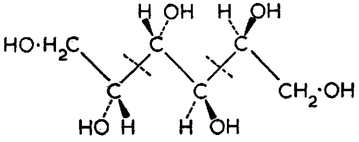


Galactitol

(XXIII)



(XXIIa)



(XXIIIa)

(- - - - Position of preferential attack by periodate)

These results, however, do not prove that such conformations are adopted in solution. The phenomenon may be due to differences in stability of the cyclic intermediates derived from the *threo*- and the *erythro*-systems. In the same way the results obtained during studies of cyclic acetal formation[45] and borate complex-formation,[46] which have been interpreted as indicating that acyclic carbohydrates take up zig-zag conformations, may be equally readily explained in terms of stability of the cyclic structures involved. Less ambiguous proof is given by Hough and Bragg[47] who showed that a large substituent R in (XX) hinders approach of reagents to position 1, and by Littleton[48] who showed by *X*-ray analysis that in a crystal the gluconate ion has a nearly planar six-membered zig-zag carbon chain.

Stereochemistry of Cyclic Intermediates and Derivatives

The most common type of cyclic structure derived from sugars is that obtained by the reaction of an α-diol in the pyranose ring with a molecule or ion to give a system of fused five- and six-membered rings. *cis*-α-Diols

---

[45] Barker, Bourne, and Whiffen, *J.*, 1952, 3865.
[46] Foster, *Adv. Carbohydrate Chem.*, 1957, **12**, 81.
[47] Hough and Bragg, *J.*, 1957, 4347.
[48] Littleton, *Acta Cryst.*, 1953, **6**, 775.

(axial, equatorial hydroxyl groups) or *trans*-α-diols (equatorial, equatorial) may react in this way. The oxygen atoms of the hydroxyl groups are the same distance apart in both cases, and the relatively high reactivity of the *cis*-diol is usually ascribed to the lower energy required to bring these oxygen atoms closer together, *i.e.*, to produce flat five-membered rings. In the case of a *cis*-α-diol a reagent which tends to produce flat rings will decrease the buckling of the pyranose ring while the puckering will be increased in a molecule having a *trans*-diol.[49]

Periodate Oxidation.[50]—Buist, Bunton, and their co-workers[51] concluded that periodate cleavage of diols proceeds through a five-membered cyclic intermediate. Working with cyclohexanediols,[52] they have shown that, when the oxidising agent is the $H_3IO_6^{2-}$ ion, the greater reactivity of the *cis*-compound may not be due to the above-mentioned "buckling effects" but rather to steric interactions which reduce the stability of the five-membered ring formed. Reaction with the ion $H_3IO_6^{2-}$ occurs essentially without distortion of the pyranose ring and a buckled ring containing iodine is formed.[52] It is probable that at the pH normally used for periodate oxidations this ion is unimportant and other species, which lead to a less buckled five-membered ring and a distorted pyranose chair, must be considered. These factors are important in discussions of the details of oxidation of glycosides and free sugars which are also oxidised in the cyclic form.[53]

In pyranoside rings *cis*-diols are also more reactive than *trans*-diols, so that mannosides and galactosides are oxidised more rapidly than glucosides.[54] A spectacular demonstration of the difference in rates of oxidation of galactosides and of glucosides and fructofuranosides (which have only *trans*-α-diols) has been provided by Mitra and Perlin[55] who preferentially oxidised the galactoside units in the tri- and tetra-saccharides, raffinose and stachyose, and isolated sucrose from the oxidation products.

The presence of substituents on a ring is important in determining the rate and site of primary oxidation. While it is uncertain where some pyranosides with small aglycone groupings suffer primary attack, it has been shown[56] that the 3,4-bond in phenyl β-D-glucoside is cleaved preferentially. The rate of oxidation is less than for the α-anomer. It was assumed[56] that these effects arise because of steric hindrance by the equatorial phenoxy-group, particularly felt at the $C_{(2)}$–$C_{(3)}$ position.

---

[49] Angyal and Macdonald, *J.*, 1952, 686.

[50] Jackson in "Organic Reactions", Wiley and Sons Inc., New York, 1944, Vol. II., p. 341; Bobbitt, *Adv. Carbohydrate Chem.*, 1956, **11**, 1.

[51] Buist and Bunton, *J.*, 1954, 1406; 1957, 4567, 4580.

[52] Buist, Bunton, and Miles, *J.*, 1959, 743.

[53] Hough, Taylor, Thomas, and Woods, *J.*, 1958, 1212.

[54] Halsall, Hirst, and Jones, *J.*, 1947, 1427; Jackson and Hudson, *J. Amer. Chem. Soc.*, 1937, **59**, 994.

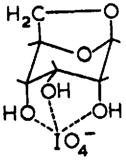
[55] Mitra and Perlin, *Canad. J. Chem.*, 1957, **35**, 1079.

[56] Garner, Goldstein, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1958, **80**, 1206.

Examination of models shows that this explanation is not entirely satisfactory, and we believe that the large equatorial aromatic substituent may cause partial locking of conformation particularly at the $C_{(1)}$ end of the sugar ring, thereby reducing the tendency of the 2- and 3-hydroxyl groups to form the planar intermediate in the oxidation; the phenoxy-group in an axial position has an influence on the conformation and flexibility of the ring which is quite different. A 6-trityl group was shown to hinder attack at position 3, 4, and the rate of oxidation at the 2,3-diol position in methyl 4-chloro-4-deoxy-D-glucoside is reduced by the presence of the chlorine atom.[57] It is not known whether these effects are caused by steric hindrance to approach of the attacking ion or to alterations in the flexibility of the pyranose rings caused by the introduction of large substituents.

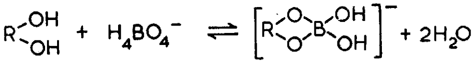
The periodate oxidation products of glycosides have been studied[58] and six-membered ring structures with appropriate conformations have been proposed.

Barker and Shaw[19] encountered a novel feature in the oxidation of D-ribose when a complex with the periodate ion involving three-point attachment was observed. Subsequently other compounds containing *cis-cis*-triols were found to form such complexes, *e.g.*, 1,6-anhydro-D-allose gives the complex (XXIV). This indicates that D-ribose may react in the 1C conformation in which one hydroxyl group of the reacting triol is equatorial and the other two are axial.



(XXIV)

Borate Interaction and Electrophoresis.[46]—It is well known that borate anions form complexes with diols of suitable stereochemical form[59] and it is believed that equilibria of the type



$$R\begin{smallmatrix}OH\\OH\end{smallmatrix} + H_4BO_4^- \rightleftharpoons \left[R\begin{smallmatrix}O\\O\end{smallmatrix}B\begin{smallmatrix}OH\\OH\end{smallmatrix}\right]^- + 2H_2O$$

are involved. The reaction is much used in carbohydrate chemistry to provide sugar molecules with an electric charge, by virtue of which they can move across a potential gradient. The rate of migration (M_G value) is dependent upon the position of the equilibria, which is governed to a

---

[57] Buchanan, *J.*, 1958, 995.

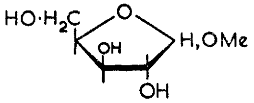
[58] Cadotte, Dutton, Goldstein, Lewis, Smith, and Van Cleve, *J. Amer. Chem. Soc.*, 1957, **79**, 691; Goldstein, Lewis, and Smith, *ibid.*, 1958, **80**, 939; Guthrie and Honeyman, *Chem. and Ind.*, 1958, 388.

[59] Boeseken, *Adv. Carbohydrate Chem.*, 1949, **4**, 189.

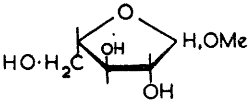
large extent by the stereochemistry of the diol. Favourably disposed hydroxyl groups form stable complexes and confer high M_G values on the sugar. The strongest complex is formed with a 1,2-*cis*-diol, although the borate ion will form complexes to a smaller degree with other diols. By determining the M_G values of a range of substituted glucoses Foster[60] has shown that at pH 10 there are three main sites of combination in glucose. These are the 2,4- and 4,6-positions of the open-chain form, and the 1,2-*cis*-diol in the α-form of either glucopyranose or glucofuranose. Although there are other possible sites of interaction, *e.g.*, the 2,3-diol, it is believed that these are relatively unimportant. It has been shown similarly[61] that the main sites of borate interaction with D-galactose are 1,2 and 3,4 in the ring form, and, less important, 4,6 in the acyclic form. Consequently the M_G value of a sugar or a sugar derivative depends on the sites available for complex formation, the stability of the different complexes formed, and presumably the ease with which the open-chain structures can be attained.

A study of the M_G values of glycosides shows that the extent of complex-formation in some cases depends upon anomeric configuration and also confirms the observation that the stereospecificity for complex-formation is not great. The borate complex formed by the methyl glucopyranosides is located at positions 4 and 6[62] and is more stable in the case of the β-derivative. Foster[46] has suggested that the relative instability of the complex obtained from methyl α-D-glucoside is due to steric interaction between the axial glycosidic methoxyl group and the axial hydrogen atoms located at positions 3 and 5.

The methyl xylopyranosides do not migrate in borate buffer, and so the high M_G values of the methyl arabopyranosides can be attributed to complex-formation by the 3,4-diol. Reaction at a *cis*-2,3-diol is hindered in pyranosides which have an aglycone grouping in a *cis*-disposition; *e.g.*, methyl β-D-lyxopyranoside, methyl β-D-mannopyranoside, and methyl α-D-gulopyranoside all form complexes less readily than do their anomers. In pentofuranosides[62] a *cis*-arrangement of the 3-hydroxyl group and the 4-hydroxymethyl group, such as is present in methyl D-xylofuranosides (XXV), permits strong borate interaction, whereas a *trans*-relation, such as occurs in the L-arabofuranosides (XXVI), allows only a weak one. Comparison of the M_G values of methyl α-D-glucofuranoside, 1,2-*O*-isopropylidene-α-D-glucofuranose, and the anomeric methyl D-



(XXV)



(XXVI)

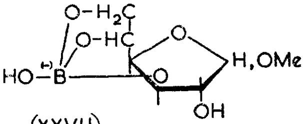
---

[60] Foster, *J.*, 1953, 982.

[61] Bouveng and Lindberg, *Acta Chem. Scand.*, 1956, **10**, 1283.

[62] Foster and Stacey, *J.*, 1955, 1778; Foster, *J.*, 1957, 1395.

galactofuranosides shows that the 2-hydroxyl substituent is not involved in the complex formed by methyl glucofuranosides. As the 3-, 5-, and 6-hydroxyl groups are important, Foster[46] has suggested that a tridentate complex (XXVII) of the type proposed by Angyal and McHugh[18] is formed.



(XXVII)

Acetals and Ketals.—For the purposes of this Review attention will be confined to the acetals and ketals formed by aldoses and aldosides. The chemistry of the acetals and ketals of glycitols has been dealt with in detail by Barker *et al.*[45] and by Mills.[63] Generally aldehydes condense with cyclic and acyclic polyhydroxy-systems to form, preferentially, six-membered rings but ketones more often give rise to five-membered rings.[64] It has been suggested[40] that factors important in this connexion are the size of the substituents in the ketone used and the fact that, if a six-membered cyclic ketal is formed, one of these substituents must assume an unfavourable axial disposition. Both factors tend to make a six-membered ring less likely for ketals.

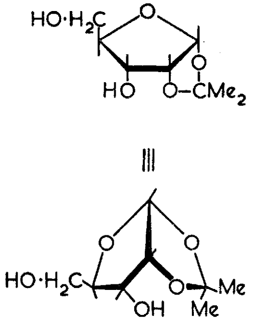
Five-membered substituent rings. (*a*) On furanose sugars. When a 1,3-dioxolan ring is fused to a furanose ring the union is generally *cis*; isopropylidene derivatives are the usual examples studied, *e.g.*, 1,2-*O*-isopropylidene-α-D-glucofuranose. Condensation may also occur with exocyclic hydroxyl groups, but on partial acidic hydrolysis the fused ring system is always the most stable part of the molecule; *e.g.*, 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose yields the 1,2-linked ketal. The bicyclic system assumes a V shape, substituents taking up *endo*- or *exo*-positions, and the favoured course of reaction is that which leads to a product with a minimum number of *endo*-substituents. For example, D-ribofuranose can afford theoretically either 1,2-*O*-isopropylidene-α-D-ribofuranose (XXVIII) or 2,3-*O*-isopropylidene-α- or -β-D-ribofuranose (XXIX) or (XXX). It is clear that (XXX), with the fewest large *endo*-substituents, will be favoured, and it is indeed formed.[65] When aldehydes are used to produce such bicyclic systems isomerism at the acetal carbon atom is theoretically possible, but interactions caused when the large alkyl or aryl grouping is in an *endo*-position ensure that only one isomer is obtained; *e.g.*, 1,2-*O*-benzylidene-α-D-glucofuranose has the structure (XXXI).

(*b*) On pyranose sugars. *cis*-Fusion of a five-membered to a six-membered ring will cause alterations in the shape of the larger one which depend upon the tendency of the smaller one to be planar. Only when this tendency

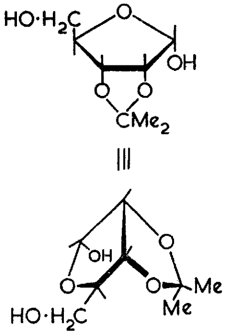
---

[63] Mills, *Adv. Carbohydrate Chem.*, 1955, **10**, 2.
[64] Barker and Bourne, *J.*, 1952, 905.
[65] Levene and Stiller, *J. Biol. Chem.*, 1933, **102**, 187.

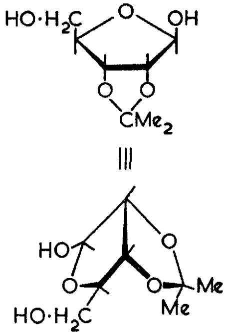
is overwhelming will the chair of the six-membered ring be distorted into a half-chair conformation,[66] and it is usually considered that five-membered acetal or ketal rings do not alter the shape of the pyranose ring radically (cf. the *cis*-perhydroindane systems[67]).



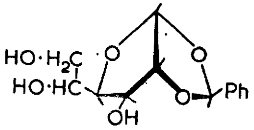
(XXVIII)



(XXIX)



(XXX)



(XXXI)

Pyranose rings on which there are two *cis*-α-diol systems, one on each side of the ring, *e.g.*, α-D-galactose, readily form diacetals in which the ring fusion is *cis-anti-cis*[68] as in 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (XXXII). It is uncertain why this structure should be more stable than the system possessing two fused five-membered rings, as in the isomer 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose. In this connexion it is noteworthy that fructose gives rise to the 2,3-*O*-benzylidene and 2,3:4,5-di-*O*-benzylidene-β-D-fructopyranoses in preference to 2,3-*O*-benzylidene-β-D-fructofuranose. Possibly the *endo*-substituents present in all these furanose derivatives hinder their formation, or perhaps this is another example of the preference of the sugars to assume pyranose ring forms. Isomerism occurs at the acetal carbon atom when a five-membered acetal ring is fused to a pyranoid ring and no preferred form can be predicted. Isomeric di-*O*-benzylidene derivatives of methyl α-D-mannopyranoside have been obtained and have been assigned[69] structures which differ only in stereochemistry at C* in (XXXIII).

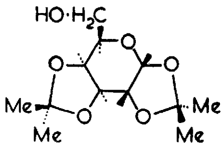
---

[66] Cf. Lemieux and Cipera, *Canad. J. Chem.*, 1956, **34**, 906.

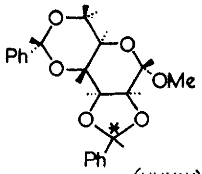
[67] Eliel and Pillar, *J. Amer. Chem. Soc.*, 1955, **77**, 3600.

[68] Turner, in "Natural Products Related to Phenanthrene", Fieser and Fieser, New York, 3rd edn., 1949, p. 620.

[69] Mills, *Chem. and Ind.*, 1954, 633; see also Dobinson, Foster, and Stacey, *Tetrahedron Letters*, 1959, **1**, 1.



(XXXII)



(XXXIII)

Six-membered substituent rings. (*a*) On furanose sugars. Although a molecule comprising a six-membered ring fused to a furanose ring is formed less readily than a system of fused five-membered rings several examples are known, *e.g.*, 3,5-acetals or -ketals of 1,2-*O*-isopropylidene-α-D-glucofuranose, 1,2-*O*-methylene-α-D-glucofuranose, 1,2-*O*-isopropylidene-α-D-xylofuranose. An explanation of the difference in the ease of acetal formation at positions 3,5 of 6-deoxy-1,2-*O*-isopropylidene-6-nitro-α-D-glucofuranose and -β-L-idofuranose[70] (which differ only in the configuration of the 5-hydroxyl group) has been offered on conformational grounds.[63] In the idose derivative the six-membered ring has no interfering axial substituents, whereas in the glucose compound both possible conformations of the largest ring have two large axial substituents on one side. It would be expected that the bulky grouping on the acetal-carbon atom would assume an equatorial position.

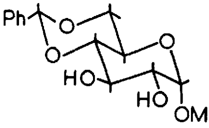
(*b*) On pyranose sugars. Six-membered acetal or ketal rings may be fused to a pyranose ring by either a *cis*- or *trans*-junction. In the latter case, provided the chair forms remain intact, the compounds are held in a specific conformation (cf. *trans*-decalin), and the hydroxyl groups of the pyranose ring are sharply defined as axial or equatorial. It is believed, however, that the end of the pyranose ring furthest from the ring junction is somewhat flexible and may be distorted during a reaction.[71] 4,6-Cyclic derivatives of pyranosides which have a *trans*-relation for the 4-hydroxyl and the 5-hydroxymethyl group fall into this class, *e.g.*, methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (XXXIV). Isomers differing in the stereochemistry at the acetal carbon atom are not found and it is assumed that the bulky group always takes up an equatorial position.

Complications arise in *cis*-fused six-membered rings. Two conformations are possible for methyl 4,6-*O*-benzylidene-α-D-galactopyranoside and these are termed "O inside" (XXXV) and "H inside" (XXXVI) respectively. Fusion of the acetal ring to the galactoside in the C1 conformation would give (XXXV) whereas reaction in the 1C form leads to (XXXVI). Less non-bonded interaction is involved in the "O inside" form which, provided other factors remain unaltered, would be expected to be more stable. The behaviour of methyl 4,6-*O*-benzylidene-α- and -β-D-galactoside in cuprammonium solution suggests that both exist in

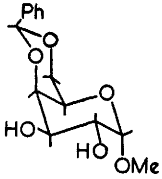
---

[70] Grosheintz and Fischer, *J. Amer. Chem. Soc.*, 1948, **70**, 1476.
[71] Korytnyk and Mills, *J.*, 1959, 636.

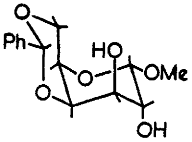
this form.[72] It would be assumed that the phenyl residue would take up an equatorial position in both the "O inside" and the "H inside" form (as shown in XXXV and XXXVI), but there is some doubt whether this applies in every case.[73]



(XXXIV)



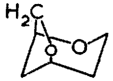
(XXXV)



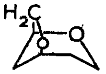
(XXXVI)

Sugar Anhydrides.[74]—All hexoses when heated in acid solution form 1,6-anhydrides to some extent. Until recently, only the pyranose forms of the anhydrides had been encountered but 1,6-anhydro-α-D-galactofuranose has now been obtained by the treatment of galactose with acid.[75] To form the anhydropyranose, the D-hexose adopts the 1C conformation[10] and a relation is noted between the conformational stabilities and the position of the equilibrium.[10,19] For example, D-glucose and D-mannose form only very small amounts (*ca.* 1%) of anhydrides, presumably because these derivatives have respectively three and two axial hydroxyl groups. On the other hand, D-idose and D-altrose, which have few instability units in the 1C conformation, exist preferentially in the anhydro-form in acid solution. Pratt and Richtmeyer[76] recently concluded that substituents at position 3 are important in determining the position of equilibrium and their prediction that a 3-deoxy-sugar would form an anhydride intermediate in amount between those produced by sugars isomeric at $C_{(3)}$ was confirmed by experiment.

Foster *et al.*[77] have considered the stereochemistry of some 3,6-anhydropyranosides and have decided that chair (XXXVII) and boat (XXXVIII) forms may both be important. 3,6-Anhydropyranosides are extremely strained and in non-aqueous acidic solution this strain is relieved by a rearrangement to the 3,6-anhydrofuranosides, *e.g.*, methyl glucopyranosides rearrange to the furanosides with retention of configuration at



(XXXVII)

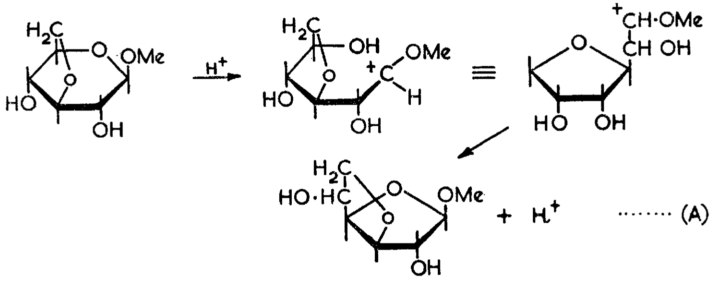


(XXXVIII)

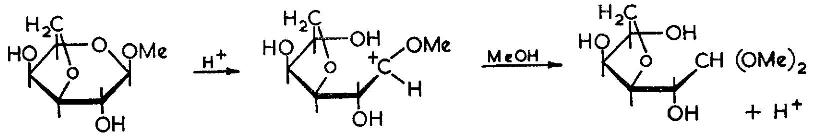
---

[72] Reeves, *J. Amer. Chem. Soc.*, 1949, **71**, 1737.
[73] Fletcher, Diehl, and Ness, *J. Amer. Chem. Soc.*, 1954, **76**, 3029.
[74] Peat, *Adv. Carbohydrate Chem.*, 1946, **2**, 37.
[75] Richtmeyer, *Arch. Biochem. Biophys.*, 1958, **78**, 376.
[76] Pratt and Richtmeyer, *J. Amer. Chem. Soc.*, 1957, **79**, 2597.
[77] Foster, Overend, and Vaughan, *J.*, 1954, 3625.

position 1 (scheme A). Alternatively, dialkyl acetals of the 3,6-anhydrides are produced if the ring-transformation is sterically impossible;[74] *e.g.*, methyl 3,6-anhydro-β-D-galactoside affords 3,6-anhydro-D-galactose dimethyl acetal (scheme B). Substitution of a phenyl for the methyl group



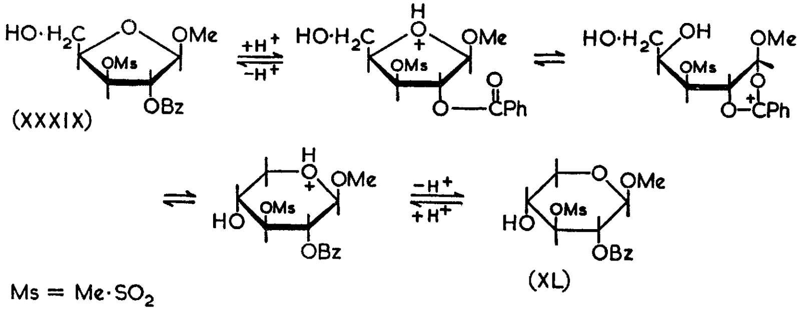
.... (A)



.... (B)

precludes such a transformation as the electrophilic character of the aromatic ring prevents the formation of the required carbonium ion.[77]

Recently the converse type of reaction has been reported,[78] namely, an acid-catalysed ring expansion of methyl glycofuranosides into the pyranosides without inversion at position 1: a derivative (XXXIX) of methyl β-D-xylofuranoside was converted into the methyl β-D-xylopyranoside derivative (XL). The mechanism proposed is as shown.



(XXXIX)

(XL)

$Ms = Me\cdot SO_2$

One of the most important classes of sugar anhydrides is the epoxides; but these have been adequately described in this series recently[79] and so are not considered here.

---

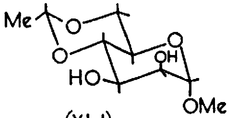
[78] Schaub and Weiss, *J. Amer. Chem. Soc.*, 1958, **80**, 4683.
[79] Newth, *Quart. Rev.*, 1959, **13**, 30.

Some Applications

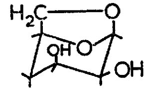
Mutarotation.—Reeves[10] has pointed out that in the equilibrium mixture of α- and β-pyranose sugars[80] in aqueous solution, the predominant isomer is that expected from a consideration of instability factors. Furthermore, the equilibrium constants lie near to a value of 2 which implies that the free energy difference between the anomers is of the order of 0·5 kcal./mole. The full implications of this are not apparent. It is interesting to note that 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-xylose, as well as lactose, conform with these observations.

Selective Reactivity of Hydroxyl Groups.[81]—After protection of the highly reactive 1-hydroxyl group in sugars by glycosidation it is found, as expected, that a primary hydroxyl group is most reactive. Of the secondary hydroxyl groups, that at position 2 often reacts before the remainder. Thus methyl α-D-glucopyranoside yielded the 6-*O*-tosyl derivative[82] and the 2,6-dibenzoate.[83]

In alicyclic chemistry it is well known that equatorial hydroxyl groups undergo esterification more readily than those which are axial.[6] Recently examples of such a difference towards esterifying agents have been furnished in the carbohydrates series. Aspinall and Zweifel[84] have demonstrated this most clearly in their experiments on mannose derivatives. For methyl 4,6-*O*-ethylidene-α-D-mannoside (XLI) and 4-*O*-methyl-1,6-anhydro-β-D-mannose (XLII) the conformations are held so that the 2- and the 3-hydroxyl groups are axial–equatorial, and equatorial–axial respectively. Selective esterification (60—70% yield) took place at the equatorial positions.



(XLI)



(XLII)

The situation is more complex in the case of methyl 4,6-*O*-benzylidene-α-D-glucoside in which both free hydroxyl groups must take up equatorial positions. It has been shown that the position of monoesterification is dependent upon the nature of the reagent.[85] Acid chlorides of carboxylic and sulphonic acids give in preponderant yield a product with the ester at position 2, anhydrides of carboxylic acids give esters at position 3, and sulphonic anhydrides again give the ester in position 2. The results listed in Table 2 were not obtained under standardised conditions but nevertheless

---

[80] "Polarimetry, Saccharimetry and the Sugars", U.S. Government Printing Office, 1942.

[81] Sugihara, *Adv. Carbohydrate Chem.*, 1953, **8**, 1.

[82] Compton, *J. Amer. Chem. Soc.*, 1938, **60**, 395.

[83] Lieser and Schweizer, *Annalen*, 1935, **519**, 271.

[84] Aspinall and Zweifel, *J.*, 1957, 2271.

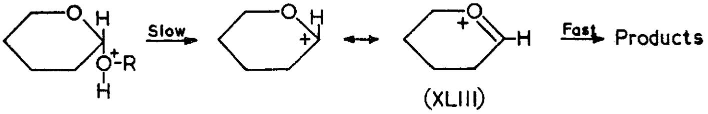
[85] Jeanloz and Jeanloz, *J. Amer. Chem. Soc.*, 1957, **79**, 2579.

indicate that the products are largely dependent upon the reagents. It is apparent therefore that esterification of a hydroxyl group in a pyranoside ring is particularly facilitated if the group is in an equatorial position but that other factors, presumably electronic, also operate.

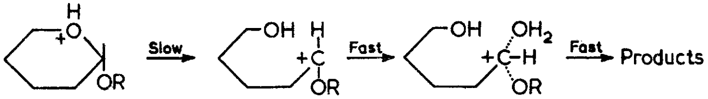
TABLE 2.

Reagent	Crystalline products (%)			
	2,3-Diester	2-Ester	3-Ester	Starting material
BzCl	35	24	6	12
$Me{\cdot}SO_2Cl$	16	68	—	—
p-$C_6H_4Me{\cdot}SO_2Cl$ ..	—	60—70[86]	—	—
Ac_2O	26	3	42	7
$(Me{\cdot}SO_2)_2O$ ..	5	36	—	22
$(p$-$C_6H_4Me{\cdot}SO_2)_2O$ ..	15	80—85	—	—

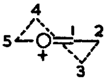
Relative Rates of Acidic Hydrolysis of Pyranosides.—The mechanism of acidic hydrolysis of glycosides has been shown[87] to follow one of the pathways A and B. There are no results available to distinguish the mechanisms. Shafizadeh[88] has pointed to the circumstantial evidence indicating that scheme B might be operative, but Edward[17] discusses the relative rates of acidic hydrolysis of pyranosides in terms of scheme A, *i.e.*, he assumes the rate-controlling step to be formation of a half-chair intermediate cation (XLIII). Formation of this H1 conformation involves



...... (A)



.... (B)



(XLIII)

small rotations about the 2,3- and 4,5-bonds. The ease of these rotations will depend in two ways upon the configuration and size of substituents on the ring: (i) it will be decreased by the opposition between substituents at positions 2 and 3 and at positions 4 and 5, and (ii) it will be assisted by

[86] Bolliger and Prins, *Helv. Chim. Acta*, 1945, **28**, 465.
[87] Bunton, Lewis, Llewellyn, and Vernon, *J.*, 1955, 4419.
[88] Shafizadeh, *Adv. Carbohydrate Chem.*, 1958, **13**, 9.

recession of the axial substituents at positions 2 and 3 from those at positions 4 and 5 respectively. The former effect explains why heptosides are more stable than hexosides, which are more stable than pentosides, and why 2,3-dideoxypyranosides are more labile than 2- or 3-deoxypyranosides, which are more sensitive than normal glycosides. It would be expected, as a result of the second effect, that glycosides with few axial hydroxyl groups in the stable conformation would be more stable than those with several, and the determined order[89] is: glucosides > mannosides > galactosides > gulosides. In the pentopyranoside series the order is xylosides > arabinosides > lyxosides.

Chromatographic Behaviour.—The mobility of carbohydrates and their derivatives on cellulose paper is dependent upon adsorption on the cellulose support and on partition between the stationary aqueous phase and the mobile organic phase. It is influenced by the fundamental properties of the molecules such as relation between the molecular weight and the number of free hydroxyl groups. In addition, other more subtle factors must operate as, in practice, it is a simple matter to separate closely related isomers.

Isherwood and Jermyn[90] have discussed structure and chromatographic behaviour and have indicated some correlations, *e.g.*, the mobilities of a homomorphous series of pyranoses show regularities so that changes in the 5-substituent (*i.e.*, H, Me, $CH_2{\cdot}OH$) cause similar effects in each configurational series. Recently[33] it has been predicted on theoretical grounds that equatorial hydroxyl groups in sugars will be considerably more strongly hydrated than axial groups. Therefore it might be expected that sugars which have few axial hydroxyl groups will be more soluble in the aqueous phase than those with several, and will consequently travel more slowly on partition chromatograms. Similarly it might be expected that the adsorption factor would reduce the relative mobility of flat molecules. Pairs of sugars differing only in configuration at a single carbon atom were compared.[90] It was shown that for hexoses a change in configuration of a hydroxyl group from above the plane of the ring to below (Haworth perspective formulæ) causes a shift in the R_F value in one direction only, and that this direction is not the same for changes at each carbon atom. The glucose–galactose pair was the only one of 12 examples which did not conform with this observation. Reconsideration of the subject shows that in every case (with the glucose–galactose exception) the change in configuration causing a decrease in R_F is an axial-to-equatorial shift when the hexoses are considered in the C1 conformation. Further, we have shown that the R_F value of a free sugar can, in most cases, be calculated from a formula of the type $R_F = K + (n + \alpha)a$ where K and a are constants for one series (*e.g.*, hexoses), n is the number of axial hydroxyls in the molecule, and α is the fraction in the

---

[89] Pigman, "The Carbohydrates", Academic Press Inc., New York, 1957, p. 209.
[90] Isherwood and Jermyn, *Biochem. J.*, 1951, **48**, 515.

equilibrium mixture of anomeric form of the pyranose sugar which has the 1-hydroxyl group in an axial position (n and α depend upon conformation and it is of interest that the conformations of the sugars which give suitable n and α values are generally those predicted by Reeves). The homomorphous series, arabinose, 6-deoxygalactose, galactose, (fructose) appears to be exceptional in that the members all have lower R_F values than would be predicted on this theory. Gulose too has a lower mobility than would be calculated and Jäger *et al.*[91] have offered an explanation for this fact in terms of a shielding of the ring by an axial hydroxyl group which prevents organic solvent molecules from approaching the ring and thus effectively increases hydration. This concept is diametrically opposed to the ideas of preferential hydration and it is difficult to see how, of the hexoses, galactose and gulose alone are subject to this effect.

Biological Considerations.—Although it is apparent that those sugars which occur most frequently in Nature have conformationally stable pyranose ring forms, and that those which are unstable conformationally do not exist extensively, there appears to be only an indirect connexion between the two features. An explanation of the phenomenon is to be found in biosynthetic terms.[92] Aldolases from plants and animals catalyse the condensation of a system having a primary alcoholic grouping with aldehydes to give a product with a diol having the D-*threo*-configuration, *e.g.*, dihydroxyacetone phosphate + D-glyceraldehyde $\rightleftharpoons$ D-fructose 1-phosphate. As a result of reactions of this type, glucose and xylose occur commonly. The occurrence of other sugars depends largely upon the ease with which they can be derived from such precursors.

The selectivity of enzymic reactions need not be stressed here; it suffices to mention the classical work of E. F. Armstrong[93] who related methyl α- and β-glucoside with α- and β-glucose by the specific use of maltase and emulsin, and the recent studies by Mayer and Larner[94] who investigated the hydrolysis of glycogen by α- and β-amylase and discussed the mechanisms in terms of conformation of the α-glucopyranose rings.

It has been demonstrated that stereochemical features are also very important in the reactions between antisera and artificial antigens having different carbohydrate determinants.[95] Such reactions are becoming increasingly useful in structural analytical studies of the polysaccharides.[96]

The authors are grateful to Drs. A. B. Foster, C. W. Rees, and J. C. P. Schwarz for valuable discussions and suggestions.

[91] Jäger, Ramel, and Schindler, *Helv. Chim. Acta.* 1957, **40**, 1310.

[92] Hough and Jones, *Adv. Carbohydrate Chem.*, 1956, **11**, 185.

[93] Armstrong, *J.*, 1903, **83**, 1305.

[94] Mayer and Larner, *J. Amer. Chem. Soc.*, 1959, **81**, 188.

[95] Marrack and Orlans, "Progress in Stereochemistry," Vol. II, ed. Klyne and de la Mare, Butterworths, London, 1958, p. 228.

[96] See Heidelberger, in "Polysaccharides in Biology", ed. Springer, Josiah Macy Jr. Foundation, New York, 1956, p. 271.