HAWORTH MEMORIAL LECTURE*

Sugar–Cation Complexes—Structure and Applications

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1 Introduction

In contrast to my distinguished predecessors in the Haworth Memorial Lectureship, I did not have the good fortune to have been associated with Sir Norman Haworth. I only met him once, when he briefly visited Australia after his retirement. But his work and his ideas on conformations, and on the importance of the conformations, of sugars were of great influence on my research. Without them the work described in this lecture would have made no sense; and if it made no sense it would not have been carried out at all. It is therefore appropriate that sugar–cation complexes should be the subject of my Haworth Memorial Lecture, and I am very grateful to the Chemical Society and to those concerned with the decision to grant me the opportunity to deliver it at this Meeting.

Since, under physiological conditions, sugars occur in solutions which also contain salts, it is relevant to query whether any association exists between these two different types of compounds. In fact, over the years, sufficient evidence had been accumulated to indicate that at least some sugars† form complexes with some cations. When this subject was reviewed[1] in 1966, no less than 172 references were cited. Nevertheless, none of the important questions had been answered: Which sugars form complexes with which cations? What are the structures of the complexes? What are the configurational or conformational features required for complex formation? What are the stability constants of these complexes?

The answers to these questions came, as they so often do, from a chance discovery followed by systematic investigation. At high pH, sugars migrate towards the anode because they are partially ionized. J. A. Mills was studying the acidity of sugars by paper electrophoresis in basic solutions[2] and was gradually lowering the pH in the hope of finding a region in which sugars were separated from each other according to their acid strength. To his surprise he found that some sugars migrated even at pH 7, but towards the cathode; this could only

*Delivered at a meeting of the British Carbohydrate Group at the University of Reading on 31 March 1980.

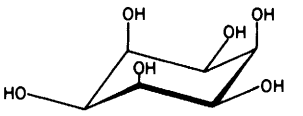
†For the purpose of this discussion the term 'sugar' will include polyols such as alditols and cyclitols. The reducing properties of the sugars are not involved in complex formation with cations.

[1] J. A. Rendleman, jun., *Adv. Carbohydr. Chem.*, 1966, **21**, 209

[2] J. A. Mills, *Biochem. Biophys. Res. Commun.*, 1961, **6**, 418.

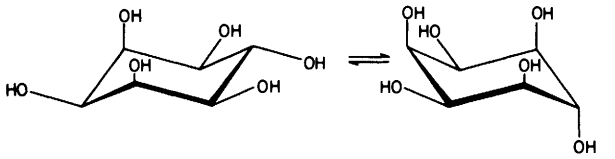
mean that they were complexed to, and migrated with, the cations. The extent of migration gave a rough measure of the complexing ability.

The polyol which showed the greatest mobility, in all electrolytes, was *cis*-inositol (1), a compound we had synthesized a few years earlier.[3] This inositol has



(1)

the rare feature of three *syn*-axial hydroxy-groups in either of its two (equivalent) chair forms, and it appeared reasonable to correlate this feature with its complexing ability. Another cyclitol which migrates well (about half as rapidly as *cis*-inositol) is *epi*-inositol, which does not have three *syn*-axial hydroxy-groups in its stable chair form (2*a*) but can provide them by flipping into the less stable one (2*b*); we had shown shortly before that this is the way in which *epi*-inositol

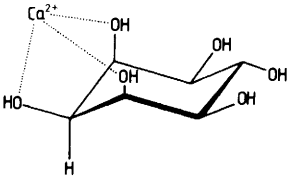


(2a) (2b)

complexes with borate ions.[4] It appeared tempting to suggest that, with cations, *epi*-inositol also complexes at the three axial hydroxy-groups of the less stable chair form.

This suggestion could not be tested until some ten years later, with the advent of n.m.r. spectroscopy. It was then shown that addition of calcium chloride to a solution of *epi*-inositol causes changes to occur in its ^{1}H n.m.r. spectrum.[5] All signals shift downfield but that of H-3 most strongly; however, there is no change in the coupling constants, showing that the conformation does not alter on complex formation. In the stable chair form there are three oxygen atoms which are close to each other, forming an almost equilateral triangle (as do three *syn*-axial hydroxy-groups), and it was postulated that these oxygen atoms, in an axial–equatorial–axial (*ax–eq–ax*) sequence, are the site of the formation of the complex (3). A quick check then showed that a number of sugars which contained an *ax–eq–ax* sequence migrated well on electrophoresis, and a number lacking such a feature did not. Similarly, the n.m.r. spectra of sugars in the

[3] S. J. Angyal and D. J. McHugh, *J. Chem. Soc.*, 1957, 3682.
[4] S. J. Angyal and D. J. McHugh, *J. Chem. Soc.*, 1957, 1423.
[5] S. J. Angyal and R. J. Hickman, *Aust. J. Chem.*, 1975, **28**, 1279.



(3)

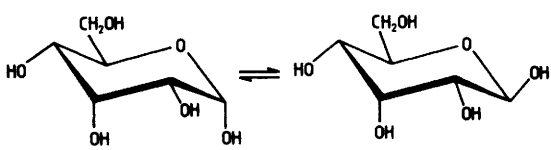
former group showed downfield shifts on addition of calcium ions, and those of sugars in the latter group did not.

2 Stability Constants

Either electrophoresis or n.m.r. can therefore be used to detect whether a complex is formed or not, but they give only a very approximate value of the stability constant of the complex. When n.m.r. is used for the evaluation of the stability constant, one has to determine the limiting shift, that is the shift which occurs when complex formation is complete; this is a good method in those cases where the stability constant is large. Since sugars complex only weakly, complete conversion into a complex is not achieved and extrapolation to the limiting shift is uncertain. By this method the approximate stability constant of the *epi*-inositol–calcium complex was found to be 3 M^{-1}.

The complexing ability of various sugars can rapidly be compared by paper electrophoresis but the result is only approximate. The rate of migration depends not only on the extent of complex formation but also on the ionic mobility of the complex, which is affected by its size and shape: compact molecules with no or few substituents migrate most rapidly.[6]

A method was then developed whereby the stability constants of one sugar with many cations could be readily measured.[7] D-Allose was chosen. Its aqueous solution consists of an equilibrium mixture of the α- (4α) and β-pyranose (4β)



(4α) (4β)

forms; only the α-form provides an *ax–eq–ax* site and therefore only the α-form complexes with cations. Addition of a cation, if it forms a complex, will therefore alter the α:β ratio by removing some of the α-pyranose from the equilibrium. The equilibrium composition is readily determined by integrating the relevant signals in the ^1H n.m.r. spectrum. It was then found that monopositive cations

[6] S. J. Angyal and J. A. Mills, *Aust. J. Chem.*, 1979, **32**, 1933.
[7] S. J. Angyal, *Aust. J. Chem.*, 1972, **25**, 1957.

changed the equilibrium only slightly, divalent ones more strongly, and tripositive ones to the greatest extent. However, only cations with an ionic radius greater than 0.8 Å complex readily; Mg^{2+} and Y^{3+}, for example, form only weak complexes. Of the common cations, Ca^{2+}, Sr^{2+}, and Ba^{2+} complex well and they have been used for most further studies. Although La^{3+} is a better complexing cation on a molar basis, it is not so when compared with Ca^{2+} weight-by-weight.

In the equilibrium solution of D-allose, small amounts of the α- and β-furanose are also present, and it was observed that the proportion of the α-furanose also increased on addition of cations to about the same extent as that of the α-pyranose.[7] It was then found that three consecutive *cis* hydroxy-groups on a five-membered ring form a complexing site almost equal to the *ax–eq–ax* arrangement.

From these experiments the stability constant of the α-D-allopyranose–calcium complex was estimated to be $\sim 6\ M^{-1}$ and that of the α-D-allofuranose–calcium complex $\sim 3\ M^{-1}$.

Stability constants have also been determined by other methods, *e.g.* by measuring the calcium ion concentration with an ion-selective electrode[5] and by determining the solubility of calcium sulphate in the presence of various sugars.[8] Some values obtained in these ways are: *epi*-inositol, 2.2; methyl α-D-ribofuranoside, 1.2; D-glucitol, 1.5; D-mannitol, 0.9 M^{-1}. The stability constants are much higher in solvents other than water, *e.g.* in methanol. (To give some appreciation of the magnitude of the stability constant: if K is 5 M^{-1}, the extent of complex formation is 64% if the concentration of both the sugar and the cation is 1 M; 27% if it is 0.1 M; 5% if it is 0.01 M.)

3 The Structure of the Complexes

N.m.r. spectra give considerable information on the structure of sugar–cation complexes. Complex formation causes a downfield shift of some proton signals, and the extent of the shift depends on the geometrical relation of the hydrogen–carbon bond to the cation. The shifts are very much greater when complexing occurs with paramagnetic cations, *e.g.* Eu^{3+} or Pr^{3+}, and then occur in both directions.[9] The greatest shift, owing to a contact effect, is of the signal of a proton in a short planar zig-zag arrangement of bonds to the cation; the shift then is *upfield* with Eu^{3+} and *downfield* with Pr^{3+}, opposite to the usual direction of the shift caused by shift-reagents. By the study of these shifts the location of the cation can usually be defined.[10]

X-Ray crystal structure analysis can often confirm the structure of a complex derived by other means. Crystalline addition compounds of sugars and various salts in stoicheiometric proportions have been known for a long time.[1] Their existence, however, does not constitute evidence that complex formation occurs to any significant extent in solution; it only indicates that the sugars, cations, and anions can fill the space in a regular packing, usually held together by hydrogen

[8] A. P. G. Kieboom, H. M. A. Buurmans, L. K. van Leeuwen, and H. J. van Benschop, *Recl. Trav. Chim. Pays-Bas*, 1979, **98**, 393.
[9] S. J. Angyal and D. Greeves, *Aust. J. Chem.*, 1976, **29**, 1223.
[10] S. J. Angyal, D. Greeves, L. Littlemore, and V. A. Pickles, *Aust. J. Chem.*, 1976, **29**, 1231.

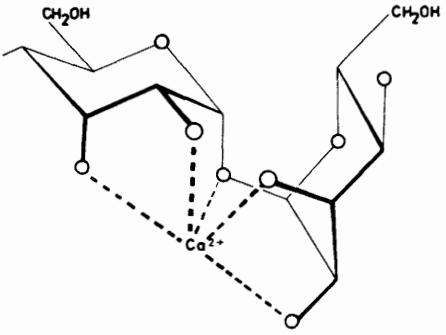
bonds and by co-ordination of cations with oxygen atoms. When dissolved in water, the hydrogen bonds will be broken and water will usually displace the hydroxy-groups from their co-ordination with the cation. Whether there will be any complex formation in solution cannot be predicted from the crystal structure. An example is the historically important sucrose $NaBr \cdot 2H_2O$, one of the first sugar structures determined by *X*-ray crystallography.[11] In solution, there is no noticeable complex formation between sucrose and sodium ions.

However, when complex formation is known to occur in solution, it is very probable that the cation will be co-ordinated to the same site in the crystal as it is in solution. Additional points of co-ordination are usually found in the crystal structure but the site where the cation is co-ordinated to three (or more) oxygen atoms is the site of co-ordination in solution. About half a dozen structures of this type have recently been determined by *X*-ray crystallography. The first[12] was that of D-mannose, $CaCl_2 \cdot 3H_2O$, which showed that the sugar is in the β-furanose form and the cation is co-ordinated to O-1, O-2, and O-3; additional co-ordination occurs to O-5 and O-6 of another mannose molecule and to the water molecules. *epi*-Inositol, $SrCl_2 \cdot 4H_2O$, shows, in the crystalline state, co-ordination of the cation to the *ax–eq–ax* sequence of oxygen atoms.[13] The conformation of the inositol is the same in the complex as in the uncomplexed molecule[14] except that the distance between the complexing oxygen atoms, and particularly between the two *syn*-axial oxygen atoms, is shorter (2.82 instead of 2.96 Å). This seems to be generally true;[15,16] apparently, complex formation reduces the interaction (*gauche* or *syn*-axial) between the participating oxygen atoms, thereby providing part of the driving force towards complex formation.

A case of particular interest is that of *allo,allo*-trehalose (α-D-allopyranosyl α-D-allopyranose), a molecule synthesized in the expectation that the two *ax–eq–ax* sequences present in its two moieties will complex simultaneously, forming a pentadentate complex. A study of n.m.r. spectra did not prove the nature of the complex but *X*-ray crystallography of the $CaCl_2$ complex[17] proved that it is, indeed, pentadentate (5). The cation is co-ordinated to O-1, O-2, O-3, O-2′, and O-3′ and, in addition, to four water molecules. Trehalose itself, having the non-complexing *gluco,gluco* configuration, crystallizes with one equivalent of calcium bromide but here the cation is co-ordinated to four different molecules.[16]

The alditols all form complexes with cations but the extent of complex formation varies greatly with their configuration. N.m.r. evidence (see below) indicates that three consecutive hydroxy-groups are involved in complex formation;[18] they have to arrange themselves, by rotation around carbon–carbon bonds, into an equilateral triangle similar to the *ax–eq–ax* arrangement. The energy required

[11] C. A. Beevers and W. Cochran, *Proc. R. Soc. London, Ser. A*, 1947, **190**, 257.
[12] D. C. Craig, N. C. Stephenson, and J. D. Stevens, *Carbohydr. Res.*, 1972, **22**, 494.
[13] R. A. Wood, V. J. James, and S. J. Angyal, *Acta Crystallogr., B*, 1977, **33**, 2248.
[14] G. A. Jeffrey and H. S. Kim, *Acta Crystallogr., B*, 1971, **27**, 1812.
[15] C. E. Bugg and W. J. Cook, *J. Chem. Soc., Chem. Commun.*, 1972, 727.
[16] W. J. Cook and C. E. Bugg, *Carbohydr. Res.*, 1973, **31**, 265.
[17] J. Ollis, V. J. James, S. J. Angyal, and P. M. Pojer, *Carbohydr. Res.*, 1978, **60**, 219.
[18] S. J. Angyal, D. Greeves, and J. A. Mills, *Aust. J. Chem.*, 1974, **27**, 1447.



(5)

for this process will depend on the configuration of the alditol chain. When three consecutive carbon atoms have the *threo–threo* configuration, the complexing site has no unfavourable interaction, and a complex is readily formed (Figure 1).

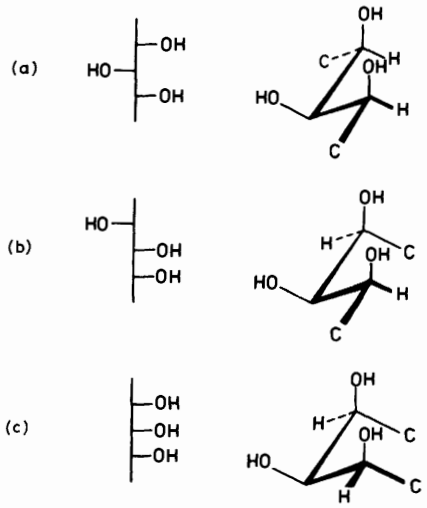


Figure 1 *The complex-forming conformation of* (a) *a* threo–threo, (b) *an* erythro–threo, *and* (c) *an* erythro–erythro *triol sequence*

An *erythro–threo* sequence produces a complexing arrangement in which there is a *gauche* interaction between two segments of the alditol chain, a somewhat less favourable arrangement. When the sequence is *erythro–erythro*, the complexing conformation has a 1,3-parallel interaction between two segments of the chain, and is unfavourable. Hence the great difference in complexing ability between iditol (all-*threo*) and allitol (all-*erythro*); the electrophoretic mobility of the

former is 0.24, that of the latter 0.09. Somewhat similar considerations apply to the terminal hydroxy-group when it is involved in complex formation.

The sites of complexing in alditols and their conformations in aqueous solution have been determined by the use of n.m.r. spectroscopy.[18] The spectra of the alditols give little information since most of the signals overlap; however, on addition of europium ions first-order analysis of all or part of the spectrum becomes possible. The strong upfield shift of the signal of one proton identifies it as being attached to the same carbon atom as the central of the three complexing oxygen atoms. The other shifts (upfield and downfield) define the conformation of the complex. The spectrum also contains information on the conformation of the uncomplexed alditol. Under the conditions used in this shift experiment, no more than about 10% of the alditol is converted into the metal complex; since the spectrum observed is the weighted average of those of the complexed and uncomplexed molecules, the coupling constants are essentially those of the uncomplexed alditol. The interesting feature of this method is that the lanthanide-induced shifts (since they occur only in the complex) provide information on the conformation of the complex, whereas the coupling constants define the conformation of the uncomplexed molecules. In this way the change of conformation on complex formation can be indirectly observed.[18]

4 Applications

A. N.m.r. Spectroscopy.—Intractable n.m.r. spectra can often be resolved by the addition of paramagnetic ions if the compound forms a complex with them.[19] Europium and praseodymium ions are particularly useful; they shift the signals in opposite directions, so that when one of them does not achieve the desired result the other one may do so. The main advantage of this method is that it is applied to aqueous solutions where the shift reagents cannot be used.

For example, all the proton signals of xylitol coincide; but gradual addition of europium nitrate to the aqueous (deuterium oxide) solution shifts the signals so that complete resolution is achieved and all the coupling constants can be determined.[18] In the ^{1}H-n.m.r. spectrum of methyl β-D-talofuranoside the signals of H-2 and H-3 overlap, even at 270 MHz; in consequence the signals of H-1 and H-4 show virtual coupling, and none of the signals is of first order. After addition of a small amount of praseodymium nitrate, the signals of H-1 and H-4 become normal and yield the coupling constants; a larger amount of the reagent causes H-2 and H-3 to separate from each other too.[20] Other examples of this method have been described.[10]

B. Paper Electrophoresis.—Paper electrophoresis is a useful technique for revealing the composition of mixtures and for identifying compounds. Once the paper electrophoresis apparatus has been set up the technique requires no more

[19] S. J. Angyal, *Carbohydr. Res.*, 1973, **26**, 271.
[20] S. J. Angyal, *Carbohydr. Res.*, 1979, **77**, 37.

(and often much less) time than paper chromatography, and the separations are frequently better. In particular, diastereomers are often widely separated.

Paper electrophoresis in a solution of calcium acetate depends on the varying extent of complex formation of different sugars with calcium ions. A wide range of mobility is found, depending on the number of hydroxy-groups, their configuration, the shape and size of the molecule, *etc.*[6] The technique is sensitive: complex formation to the extent of less than 1% gives rise to measurable mobility. The mobility (M_1) is expressed in comparison with that of *cis*-inositol, the fastest-moving polyol.

The electrophoretic mobilities of some 150 sugars in a buffered calcium acetate solution have been determined under standard conditions.[6] The presence of even two *cis* hydroxy-groups endows a compound with some mobility. Several *trans* hydroxy-groups in a six-membered ring also cause slight mobility. The only sugars which do not move at all are those containing only *trans* hydroxy-groups in five-membered rings. Considerable mobility is observed, of course, for compounds which have an *ax–eq–ax* sequence of three hydroxy-groups on a six-membered ring or a *cis–cis* sequence on a five-membered ring. If one of the hydroxy-groups is replaced by a methoxy-group the mobility is lowered by about one third. The electrophoretic mobility therefore gives a rapid indication of the possible structure and configuration of a sugar.

The four diastereomeric ketohexoses, for example, are well separated; the M_1 values of psicose, tagatose, fructose, and sorbose are 0.28, 0.14, 0.07, and 0.03, respectively. Psicose has an *ax–eq–ax* sequence in either of its pyranose forms, tagatose only in the (less stable) β-pyranose form, and fructose has two *cis* pairs of hydroxy-groups, sorbose only one, in one of its pyranose forms. Therefore, when a commercial sample of D-tagatose was suspected to be impure, the presence of a very slowly moving spot indicated that the impurity could be D-sorbose.

Table 1 shows the electrophoretic mobilities of some methyl glycosides. In

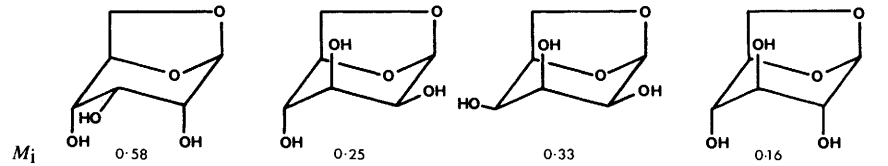
Table 1 *Electrophoretic mobilities* (M_1) *of some methyl glycosides in* 0.2M *calcium acetate*

Methyl	*Mannoside*	*Lyxoside*	*Alloside*	*Guloside*
α-Furanoside	0.05	0.07	0.22	0.22
β-Furanoside	0.27	0.30	0.07	0.08
α-Pyranoside	0.04	0.02	0.24	0.26
β-Pyranoside	0.04	0.11	0.04	0.03

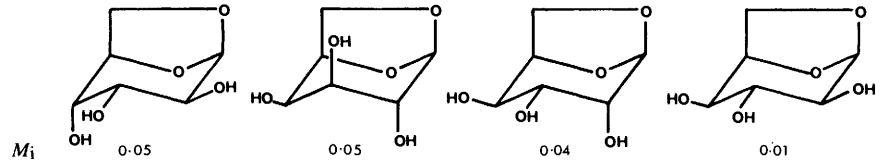
many cases, all the four methyl glycosides of a sugar are separated by paper electrophoresis. By this method, therefore, the progress of the glycosidation reaction can be followed and any subsequent fractionation or purification can be controlled.

The variation in electrophoretic mobilities is well illustrated by the eight diastereomers of the 1,6-anhydrohexoses, most of which can be separated from

each other by paper electrophoresis.[10] The *allo* isomer (6) has an *ax–eq–ax* sequence of hydroxy-groups and therefore the highest mobility. The *manno* and *talo* isomers, (7) and (8), also have three oxygen atoms in an *ax–eq–ax* sequence but one of them is part of a ring and therefore has lower electron density; the mobility is somewhat less. The *galacto*, *gulo*, and *altro* isomers, (10), (11), and (12), have one *cis* pair of hydroxy-groups, giving low mobility, and in the *ido* isomer (13) all the hydroxy-groups are *trans*, on a rather rigid ring; there is practically no mobility.

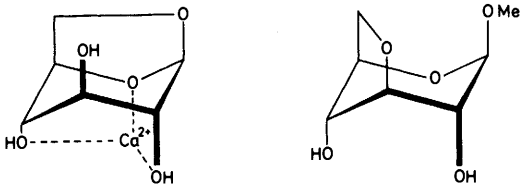


(6) (7) (8) (9)



(10) (11) (12) (13)

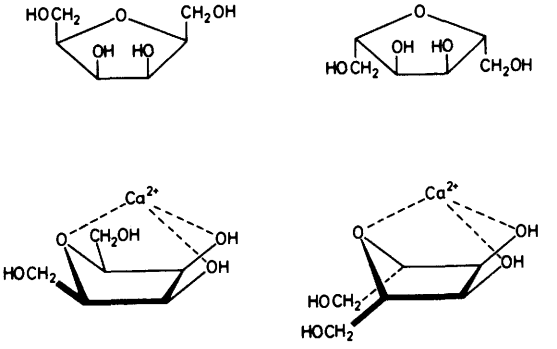
The considerable mobility of 1,6-anhydro-β-D-glucopyranose (9) must be caused by complex formation involving the two axial hydroxy-groups and the ring oxygen atom (14). These three oxygen atoms are in the same relationship as those of an *ax–eq–ax* sequence. Two *syn*-axial hydroxy-groups, in themselves, do not give rise to such mobility. The chemical shifts caused in the n.m.r. spectrum of the anhydro-glucose on addition of lanthanide ions is best interpreted as involving co-ordination to the ring oxygen atom. Surprisingly, however, methyl 3,6-anhydro-β-D-glucopyranoside (15) and its α-anomer show very little



(14) (15)

mobility, although they contain the same arrangement of *syn*-axial hydroxy-groups and the ring oxygen atom.[6] The reasons for this different behaviour is not understood.

There are other instances known of participation by the ring oxygen atom in complex formation.[21] To understand this phenomenon better, two model compounds were synthesized and studied: 2,5-anhydroallitol and 2,5-anhydrogalactitol.[22] The latter forms a reasonably strong complex with calcium ions, the former does not. The explanation is provided by the stabilities of the conformations of the complexes; that of 2,5-anhydrogalactitol (16) is favourable but that of 2,5-anhydroallitol (17) has an unfavourable interaction between the two hydroxymethyl groups and hence will not be formed to a significant extent.



(16) (17)

C. Chromatographic Column Separation.—Paper electrophoresis can be scaled up, to provide preparative separation, by the use of an ion-exchange column in the calcium form. Such a column is essentially a column of calcium ions, held in place by the ion-exchange resin. The stronger the complex formation of a polyol with calcium ions, the more its passage through the column will be retarded.

A similar column in the barium form was described in 1960 by Jones and Wall[23] to give satisfactory separation of many sugars and polyols from each other. However, the column was not used during the subsequent 15 years, probably because it was not understood how it worked, and hence it was not possible to predict what compounds it would separate. Now that the basis of the separation is understood, such a column is found to have many advantages:[24]

(1) The extent of the separations achieved can be predicted by looking at the available complexing sites or, even better, by running a paper electrophoretogram in a calcium buffer. With few exceptions, the order of elution from the column is the same as the order of the electrophoretic mobilities.

(2) Water only is used as eluant.

(3) Since the retention on the column is not due to surface absorption but to a

[21] S. J. Angyal, D. Greeves, and V. A. Pickles, *Carbohydr. Res.*, 1974, **35**, 165.
[22] S. J. Angyal and Y. Kondo, *Aust. J. Chem.*, 1980, **33**, 1013.
[23] J. K. N. Jones and R. A. Wall, *Can. J. Chem.*, 1960, **38**, 2290.
[24] S. J. Angyal, G. S. Bethell, and R. J. Beveridge, *Carbohydr. Res.*, 1979, **73**, 9.

chemical reaction, the capacity of the column is high. For compounds which complex well (*e.g.* those which have an *ax–eq–ax* sequence of hydroxy-groups) three equivalents of calcium ions are sufficient to give satisfactory retention.

(4) The column is permanent and requires no regeneration; once all components of a mixture have been eluted, the column is ready for the next separation.

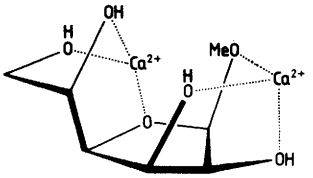
Some examples of column separations are given herewith.[24] D-Talose was a rather inaccessible sugar until Bílik[25] and his co-workers discovered that aldoses can be epimerized on C-2 by heating them in aqueous solution with a catalytic amount of molybdic acid. In this way D-galactose is converted into D-talose until equilibrium is reached; at this stage the mixture contains only 16% talose but this is readily isolated by the use of a calcium column. A batch made from 50 g of D-galactose can be separated in one passage through a 100 × 2.5 cm column of Dowex 50-W X-4 resin in the calcium form.

Sugars which form complexes with cations can be readily isolated from natural sources by the use of a calcium column.[23] Impurities can be removed; *e.g.* commercial D-tagatose, which contains D-sorbose, is quickly purified by passage of its solution through a calcium column. Recrystallization does not remove the impurity. A minor product of a reaction can be easily isolated if it forms a metal complex. For example, in the Fischer glycosidation of D-mannose, the methyl β-furanoside is a minor product but can be quickly separated from the other glycosides which do not form complexes with cations.[26] The column has industrial applications too; D-mannitol is separated from D-sorbitol by this method, and also D-fructose from D-glucose. The separations are improved by the addition of methanol or ethanol to the eluant and by running it at a lower temperature.[24]

In a few instances it was noted that a substance emerged from a calcium column much later than expected from consideration of its electrophoretic mobility.[24] In such cases, *e.g.* methyl α-L-gulofuranoside and D-*glycero*-L-*talo*-heptose, the molecule has a strong complexing site in the ring, and also a weak complexing site on the side-chain, both on the same side of the molecule. It was postulated that, when the sugar is attached to the surface of the ion-exchange resin by co-ordination with a calcium ion, a neighbouring calcium ion on the surface can form an additional complex, even with an otherwise weakly complexing site [see, for example, the structure (18) postulated for the complex of methyl α-L-gulofuranoside]. This phenomenon is denoted as 'double complexing'.

D. Applications to Synthesis.—In a reversible reaction which leads to an equilibrium of several compounds, the outcome of the reaction can be changed by the addition of cations to the mixture, if the reaction products have differing complexing ability.[27] A prime example is the Fischer glycosidation of sugars, with methanol and a strong acid, which leads to a mixture of the methyl furanosides

[25] V. Bílik, W. Voelter, and E. Bayer, *Annalen*, 1974, 1162.
[26] S. J. Angyal, C. L. Bodkin, and F. W. Parrish, *Aust. J. Chem.*, 1975, **28**, 1541.
[27] M. E. Evans and S. J. Angyal, *Carbohydr. Res.*, 1972, **25**, 43.



(18)

and puranosides. Addition of calcium chloride to the reaction mixture will increase the proportion of those pyranosides which have an *ax–eq–ax* sequence, and of the furanosides which have three consecutive *cis* oxygen atoms. These are usually minor components in the equilibrium mixture but they can be made the major products by the use of large amounts of calcium chloride. Fortunately, calcium chloride is very soluble in methanol. The products are then separated on a calcium column.

Several methyl furanosides have been prepared[24,28] in this way in yields considerably higher than those recorded in the literature (Table 2). The increase

Table 2 *Yields of methyl glycosides synthesized in the presence of calcium chloride*

Methyl glycoside	*Yield ref.* 28/%	*Yield lit.*/%
α-D-Ribofuranoside	69	4
β-D-Lyxofuranoside	31	3—5
β-D-Lyxopyranoside	49	2
β-D-Mannofuranoside	49	2.5
β-D-Talofuranoside	54	—

in yield, spectacular in some cases, is due not only to the shift in the equilibrium but also to the separation, without substantial losses, of the desired component by the calcium column. In the synthesis of methyl β-D-mannofuranoside, complexing is used for a third time when the compound is isolated as its readily crystallizing complex with calcium chloride.[26]

Reaction conditions have been established under which any of the four methyl D-allosides can be prepared in good yield from D-allose in one step, by varying the temperature and the concentration of acid, in the presence or absence of strontium chloride.[27] When none of the methyl glycosides forms a complex, the dimethyl acetal of the open-chain form can sometimes be obtained in the presence of calcium chloride.[29] Normally this acetal is formed in only very small amounts.

Other reactions may also be affected by the presence of complexing cations. Methyl furanosides which complex readily are hydrolysed at a slower rate in the

[28] S. J. Angyal, C. L. Bodkin, J. A. Mills, and P. M. Pojer, *Aust. J. Chem.*, 1977, **30**, 1259.
[29] F. W. Parrish, S. J. Angyal, M. E. Evans, and J. A. Mills, *Carbohydr. Res.*, 1975, **45**, 73.

presence of acids than those which do not.[30] The first step of the hydrolysis, protonation by the acid, is impeded by the positive charge of the glycoside–cation complex. This retardation may be useful for the selective hydrolysis of polysaccharides.

5 Biological Implications

At the beginning of these studies it was thought that complex formation between sugars and cations could have important biological implications. This now does not appear to be the case, at least as far as simple sugars are concerned. For substantial complex formation to occur, concentrations of sugars and cations are required which are higher than those usually found in living organisms. Moreover the sugars which readily form complexes (*e.g.* allose, talose, ribose) rarely occur in Nature; those which are common do not complex well.

On the other hand, complexing is more likely to occur on the surface of polysaccharides, especially on cell walls; reaction on a surface corresponds to one occurring at high concentration. Polysaccharides may also offer an opportunity for complexing to more than three oxygen atoms (a simple model compound is α-D-allopyranosyl α-D-allopyranoside[17]). Alginic acid is a relevant example. This polysaccharide is of commercial importance because it forms strong gels in the presence of calcium ions. The molecule of alginic acid is a long chain, consisting of β-D-mannuronic acid and α-L-guluronic acid residues. It is well known that the higher the proportion of guluronic acid, the better are the gel-forming properties of alginic acid.[31] α-Hydroxy-acids all form complexes with calcium ions but α-guluronic acid can also complex at an *ax–eq–ax* site; combination of these two modes of complexing is probably responsible for gel formation.[32] Figure 2 shows a possible structure of a section of the poly-

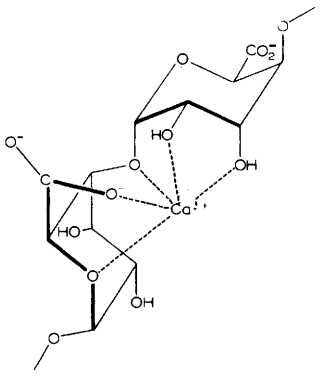


Figure 2 *Possible structure of a section of alginic acid complexed with calcium ion* (Reproduced by permission from *Pure Appl. Chem.*, 1973, **35**, 145)

[30] H. Lönnberg and A. Vesala, *Carbohydr. Res.*, 1980, **78**, 53.
[31] A. Haug and O. Smidsrød, *Acta Chem. Scand.*, 1970, **24**, 843.
[32] S. J. Angyal, *Pure Appl. Chem.*, 1973, **35**, 131.

saccharide–calcium complex; such a structure would cause the chain to be rigid. Alternatively, the cation may complex with the carboxy-group of one chain and the *ax–eq–ax* sequence of another one; gel formation would then be due to cross-linking of chains by the cation.

Quite recently, Misaki and co-workers[33] isolated from a soil bacterium a polysaccharide which forms an insoluble brittle gel in the presence of calcium ions. The polysacchride contains D-allose units besides some of the common sugars. The structure of the polysaccharide has not yet been published but it is tempting to suggest that the D-allose units occur in the α-form, thereby providing *ax–eq–ax* sites for complex formation, and therefore cross-linking.

Most of the work on complex formation with cations has so far been carried out with monosaccharides; the behaviour of polysaccharides has not yet been systematically investigated. Some interesting facts may yet await discovery.

In 1929, after having studied the shapes of sugar molecules with the aid of models, Haworth wrote[34] that 'these considerations open up a large field of inquiry into the conformations of groups as distinct from structure or configuration.' Not all of this 'large field of inquiry' has yet been explored.

[33] A. Misaki, Y. Tsumuraya, M. Kakuta, H. Takemoto, and T. Igarashi, *Carbohydr. Res.*, 1979, **75**, C8.

[34] W. N. Haworth, 'The Constitution of Sugars', Edward Arnold and Co., London, 1929, p. 90.