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Topics in carbohydrate stereochemistry

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Abstract - In its crystal form, D-glycero-L-allo-heptitol is in the $_3G^-$ form which has a 1,3-parallel interaction between an oxygen and a carbon atom. In solution, the heptitol and its acetate also assume this conformation. Such an interaction, not previously reported for an alditol, has also been found to occur in several other straight-chain sugar derivatives.

Characteristic differences were found between the R_F values of carbohydrates on ion-exchange t.l.c. plates in the calcium and in the lanthanum form. Alditols which have a xylo sequence of three hydroxyl groups complex better with La(III) ions; methyl glycosides complex better with Ca(II) ions.

In the Koch-Stuart method of replacing carbon-bound hydrogen atoms with deuterium over Raney nickel, some hydrogen atoms exchange slower than do others, depending on their environment and steric hindrance. Use has been made of these differences in order to prepare selectively deuterium-labelled sugars.

INTRODUCTION

It is now, in 1986, fifty years since I began research on carbohydrates (ref. 1). The problems then were not very different from those we have to tackle now but the methods available for solving them were very primitive compared with to-day's. I recall that it took me the best part of three months to determine a simple structure; nowadays, it could be finished in one or two days. I do not wish to imply that carbohydrate chemists have it easy to-day; as the methods improve, the remaining problems grow more and more difficult. The challenge remains approximately the same.

During those fifty years I have frequently been described as a carbohydrate chemist. If I were asked for a proper classification, I would prefer to be known as a stereochemist. I have always been fascinated by steric relationships in chemistry, and I found carbohydrates a particularly suitable hunting ground for my interests. Hence the three subjects of my lecture to-day, though unrelated to each other, have one thing in common: they each involve steric aspects of carbohydrate chemistry.

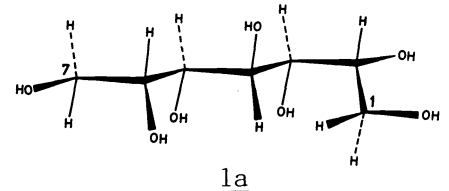
CONFORMATION OF ALDITOLS

Fifty years ago the alditols were generally believed to be preponderantly in conformations having an extended planar zigzag arrangement of carbon atoms, like that of the straight-chain hydrocarbons, though there was no evidence to support this belief. Work in the 70's, however, mainly by D. Horton and his co-workers (ref. 2), has shown that the acyclic polyols and their derivatives assume this conformation only when no 1,3-parallel interaction then occurs between oxygen atoms. If such interactions are present in the zigzag form, one (or several) 120° rotations around carbon-carbon bonds occur to avoid them, resulting in a "bent" or "sickle" conformation. Thus, mannitol and galactitol are preponderantly in the planar zigzag form, glucitol, altritol and iditol have one, and allitol has two gauche arrangements in its most stable conformation. It appeared, at this stage, that the conformations of alditols no longer presented a problem.

In 1974, however, J. A. Mills (ref. 3) pointed out that, for a chain of more than six carbon atoms, there will be diastereomers which have no conformation free from 1,3-parallel interactions. He defined the circumstances under which such a condition would occur: thus, no alditol having a sequence of three consecutive centres of ribo configuration that is separated from each end of the chain by at least one asymmetric centre can avoid having a 1,3-parallel interaction. By the study of stereomodels, and from theoretical considerations, Mills predicted the most probable conformation for each of the ten heptitols; three of these would have 1,3-parallel interactions between oxygen atoms.

To test these predictions, we decided to investigate, in collaboration with R. Le Fur in Grenoble, the conformations of the heptitols in solution (ref. 4). The ideal method for this purpose would have been ^{1}H-n.m.r. spectroscopy but the H signals of the alditols are very close to each other and could not be resolved by the spectrometers then at our disposal. The ^{1}H-n.m.r. spectra of the hexitols were fully analysed only in 1984 (ref. 5) by the use of a 400 MHz spectrometer, computer simulation and deuterium labelling. Hence we used ^{13}C-n.m.r. spectra for the heptitols,having first explored this method with the hexitols (ref. 6). The information thus obtained is not as conclusive as that given by proton spectra but the results appeared to confirm Mills's predictions (ref. 3).

In those instances where 1,3-parallel interactions occur between hydroxyl groups, this repulsive interaction could be reduced in the crystalline state by an intramolecular hydrogen bond. Such a bond is rare in acyclic polyols, and another example would have been welcome. It was of interest therefore to determine whether it occurred in the crystals of D-glycero-L-allo-heptitol, one of the heptitols for which such a conformation was postulated. An X-ray crystal structure determination was carried out (ref. 7) and showed (Fig. 1), to our surprise, that in the solid state this heptitol did not assume the postulated conformation (1a), with a 1,3-parallel interaction between two hydroxyl groups, but a conformation (1b) which has such an interaction between an oxygen and a carbon atom (C-2 and O-5).



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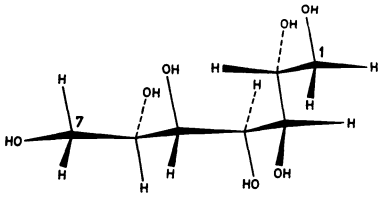

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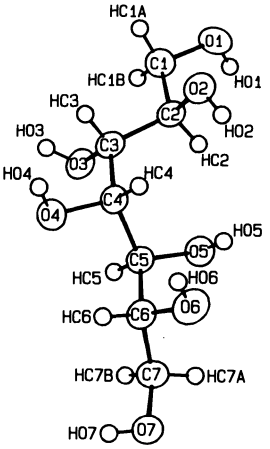


Fig. 1. The crystal structure of D-glycero-L-allo-heptitol

The conformation found in the crystal is not necessarily the same as the one preponderant in solution; in the crystal, packing forces and intermolecular hydrogen bonds may determine the conformation. Hence it appeared imperative now to determine the solution conformation of D-glycero-L-allo-heptitol. At 300 MHz, with the use of the 2D-COSY technique and a 2D J-resolved spectrum, all the coupling constants were determined, and they showed that in aqueous solution, also, the $_3G^-$ form (1b) is preponderant (ref. 7).

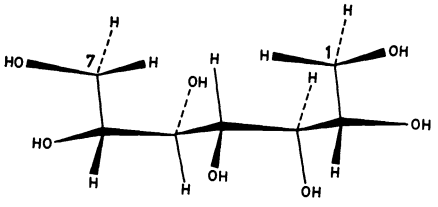
It must be emphasized that neither our group, nor Mills, nor Horton have considered, for the alditols, conformations with a 1,3-parallel interaction between an oxygen and a carbon atom (usually designated as C//O); it has been tacitly assumed that they would be too unstable to be present in substantial proportions. Hence,the conformation of D-glycero-L-allo-heptitol proved to be puzzling, and thereafter we were looking at similar systems for the possible presence of C//O interactions.

Le Fur and his colleagues in Grenoble then determined the conformation of the heptaacetate of D-glycero-L-allo-heptitol: this, also, proved to be mainly in the $_3G^-$ form (1b). Analysis of the ^{1}H-n.m.r. spectrum was again difficult but was solved by the use of heteronuclear ^{13}C-^{1}H correlation by the Reverse DEPT method (ref. 8). A more surprising result was obtained for the heptaacetate of D-glycero-D-gluco-heptitol. The free heptitol, in deuterium oxide, is mainly in the $_2G^-$,$_5G^+$ form (2), as postulated by Mills; the preponderant conformation of the heptaacetate, however, is $_3G^+$,$_4G^+$ (3) which has a 1,3-parallel interaction between C-3 and O-6. In this case, therefore, a conformation with a C//O interaction is preferred to one which has no 1,3-parallel interaction at all.

It was further found that meso-glycero-allo-heptitol is also preponderantly in conformations which have C//O interactions, rather than in the form assigned to it by Mills, which would have an O//O interaction.

A search of the literature subsequently disclosed several other instances of 1,3-parallel interactions between carbon and oxygen atoms in acyclic sugar derivatives. Even in a simple hexitol they were encountered: only about half of the molecules of allitol are in

the conformation ($_2\underline{G}^-$,$_4\underline{G}^+$) which is free of such interactions; the other half assumes two (enantiomorphous) conformations which have C//O interactions. Mostly these interactions were found in molecules where the alternative conformations would have an O//O interaction but occasionally the conformation with a C//O interaction is preferred to another one which would have no 1,3-parallel interaction at all. The reason for this behaviour is not understood.



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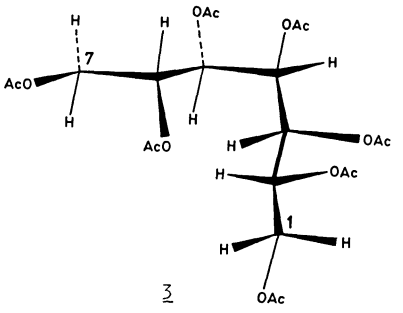

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In acyclic compounds, apparently, the C//O interaction is not as prohibitive as had been thought (ref. 3). In fact, this interaction may be quite small. In the crystal structure of D-glycero-L-allo-heptitol (Fig. 1), the distance between C-2 and O-5 is 3.06 Å; the C-3—C-2 and the C-5—O-5 bonds are not truly parallel to each other. This is the result of a widening of the bond angles on C-3 and C-4, and of an alteration by about 15° from the fully staggered arrangement along the C-3—C-4 bond. It is not clear,however, why such deviations from the usual angles favour C//O interactions over O//O interactions.

As a result of these studies we now know much more about the conformations of alditols but understand much less.

SEPARATION OF SUGARS AND POLYOLS ON ION-EXCHANGE T.L.C. PLATES IN CALCIUM AND LANTHANUM FORMS

Sugars and polyols can often be separated readily on a preparative scale by chromatography on columns containing a cation-exchange resin in the calcium or barium form (ref. 9). This method is not used as much as it deserves to be; yet, it has many advantages: (i) Separation is rapid and clean, and the recoveries are nearly quantitative; (ii) the eluant is water only; (iii) in favourable cases, the capacity of the column is large; (iv) the column is stable and need not be regenerated; and (v) the sequence of the emergence of components from the column can be predicted by paper electrophoresis of the mixture (ref. 10). The separation is due to formation of complexes between sugars and the cation; the stronger the complex, the longer the sugar is retained on the column.

A similar separation, on the analytical scale, is frequently performed by h.p.l.c.; the column marketed under the name of "Sugar Pak" contains a cation-exchange resin in its calcium form. The close correlation between retention on this column (ref. 11) and electrophoretic mobility in a calcium acetate solution (ref. 10) is shown in Fig. 2. Some of the sugars (indicated by name on the graph) show exceptional behaviour but this is readily explained. These sugars (e.g., D-allose) are present in aqueous solution as mixtures of tautomeric forms, those capable of complex formation (the α-pyranose and the α-furanose) being only minor components. Hence the sugar behaves on the column like a weakly complexing compound. In paper electrophoresis, however, the sugar is dissolved in a solution containing a calcium salt, and complex formation with the cation shifts the tautomeric equilibrium (ref. 12) in favour of those components which form complexes readily. Hence the sugar behaves like a strong complexer.

Paper electrophoresis is an easy and rapid method of analysis but the required apparatus is not available in every laboratory. Hence it is fortunate that, for the present purpose, it can be replaced by a different method not requiring special apparatus: thin-layer chromatography (t.l.c.) on ion-exchange resins. Weigel (ref. 13) first applied this method to carbohydrates. Plates coated with a mixture of a cation-exchange resin and silica gel are now commercially available (Polygram Ionex-25 SA, manufactured by Macherey-Nagel GmbH, 5160 Düren, Germany) and can be readily converted into the calcium form by simply immersing them in a solution of a calcium salt. The plates are developed with water; the stronger the complex formation, the lower will be the R_F of the compound.

The behaviour of a polyol on paper electrophoresis and on t.l.c. plates is not always analogous (ref. 14). The electrophoretic mobility (M_j) depends not only on the extent of complex formation but also on the shape and size of the molecule which migrates in the electrolyte. T.l.c. is more closely analogous to column chromatography, and in most cases provides better advance information on its outcome.

In a recent paper, Petruš et al. (ref. 15) reported that alditols are better separated on a column in its lanthanum than in its calcium or barium form. They also found that on such a column D-glucitol was retained much longer than D-talose, whereas on a calcium column the opposite was true. It appeared therefore of interest to investigate whether the advantages of lanthanum columns would extend to other types of compounds, and also to ascertain to what extent complexing with lanthanum differs from complexing with calcium.

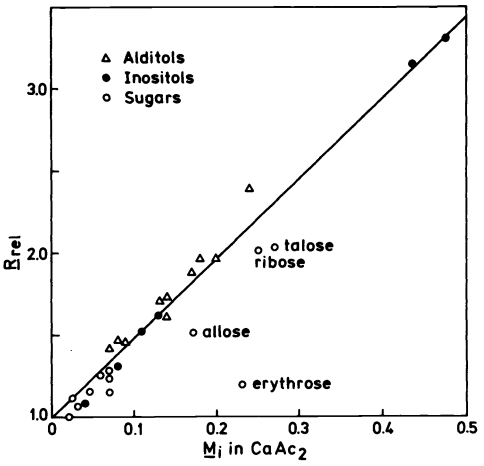


Fig. 2. Comparison of electrophoretic mobilities in calcium acetate with relative retention times (glucose = 1) on a Sugar Pak 1 column.

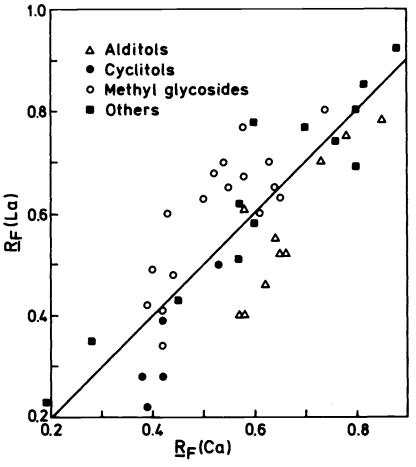


Fig. 3. Comparison of R_F values on calcium t.l.c. plates with R_F values on lanthanum plates.

For this investigation, we used the t.l.c. technique: we tested numerous sugars, methyl glycosides, anhydro-sugars, inositols and alditols. A comparison, shown in Fig. 3, of the R_F values on the calcium and on the lanthanum plates shows that they are close to each other in some cases but differ by as much as ±0.2 in others, the difference occurring with abour equal frequency in either direction. If the R_F values were the same on the two types of plates, the points would all fall on the straight line shown in Fig. 3. Two groups of compounds can be recognized as behaving differently with the two different cations.

ALditols having a xylo sequence of three hydroxyl groups (e.g., xylitol, glucitol) form stronger complexes with lanthanum than with calcium ions. This is also shown by their electrophoretic mobilities (ref. 14) which are about twice as large for La(III) as for Ca(II) ions in these cases. Alditols not having such an arrangement have about the same mobility with either cation. The preponderant conformation of alditols with a xylo sequence is a sickle form (see above) which has to change into the planar zigzag in order to form a complex with the cation (ref. 16). Apparently the tripositive La(III) ion has greater propensity to induce this change than the dipositive Ca(II) ion.

Methyl glycosides in which O-1 is involved in complex formation (e.g., methyl α-D-allo-pyranoside and -furanoside) have higher R_F values on lanthanum than on calcium plates. Apparently complex formation with lanthanum is more susceptible to steric hindrance by the methyl group, probably because lanthanum is coordinated to more oxygen atoms than calcium. Glycosides in which O-1 is not involved in complex formation have approximately the same R_F values on the two plates; examples are the methyl α- and β-D-talopyranosides (which complex at O-2, O-3 and O-4) and methyl α-D-lyxofuranoside (which complexes at O-2, O-3 and O-5). This is in accordance with the results of Lönnberg et al. (ref. 17) which were puzzling at the time they were published; they found that with calcium ions α-D-lyxofuranoside complexes much more weakly than α-D-ribofuranoside, but with lanthanum ions this order is reversed. 1,6-Anhydro-β-D-glucopyranose belongs to this group: it complexes more weakly with lanthanum ions, presumably because O-5 is hindered (ref. 17a). Complex formation occurs on O-2, O-4 and O-5.

It appears then that the main advantage of lanthanum over calcium in chromatography is, as already stated by Petruš et al. (ref. 15), in the separation of alditols. For example, the important separation of D-glucitol from D-mannitol is much better on a lanthanum than on a calcium column. There may be advantages in the separation of glycoside too; e.g., D-ribose can be separated from all four of its methyl glycosides on a lanthanum column whereas on a calcium column it coincides with its methyl α-furanoside.

A somewhat similar method has recently been described by Bilisics and Petruš: they separate polyols by chromatography on O-(carboxymethyl)cellulose paper in the lanthanum, calcium and barium forms (ref. 18). The separations are based on a combination of complex formation and phase distribution; again, lanthanum gives the best separations.

SELECTIVE DEUTERATION

A convenient method has been described by Koch and Stuart (ref. 19) for the introduction into carbohydrates of deuterium atoms bonded to carbon atoms. It consists of heating an appropriate carbohydrate derivative in deuterium oxide with a deuterated Raney nickel catalyst. In most cases, only hydrogen atoms bonded to carbon atoms having a free hydroxyl group undergo exchange (ref. 20); the method has been used mainly for the complete exchange of all such hydrogen atoms. The work of Perlin and co-workers (ref. 20) and of Barker and co-workers (ref. 21) demonstrated that various hydrogen atoms in a molecule are exchanged at different rates under these conditions. In particular, equatorial hydrogen atoms in a pyranose ring exchange faster than axial ones, and a hydrogen atom syn-axial with an oxygen atom in a pyranose ring exchanges particularly slowly. In methyl glycosides, the methoxyl group hinders the exchange reaction of the hydrogen atom in the adjacent position.

It appeared to us possible that carbohydrate derivatives could be chosen so that they are deuterated, by this procedure, selectively in only one or two positions. We therefore undertook a study of the effects of steric relationships and neighbouring substituents on the rate of exchange. The extent of the exchange was monitored by ^{1}H- and ^{13}C-n.m.r. spectra. We set ourselves the goal of finding conditions under which some hydrogen atoms are exchanged to an extent of at least 90% while the others are exchanged less than 10%. To achieve this, a ratio of reaction rates of at least 20:1 is required.

Inositols

As model compounds we chose, initially, the inositols (as we have done for many other investigations in the carbohydrate field) to avoid, at this stage, the effect of a hetero atom in the ring. Moreover, inositol methyl ethers were available to represent most of the possible arrangements of the methoxyl group and its two hydroxyl neighbours.

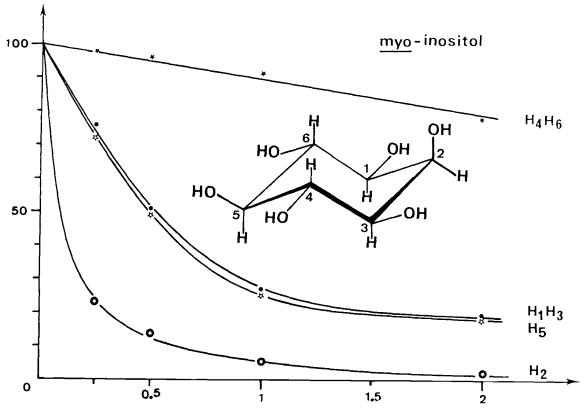


Fig. 4. The exchange of the hydrogen atoms (%) of myo-inositol at 60° versus time (hours).

The exchange of the hydrogen atoms in myo-inositol is shown in Fig. 4 (ref. 22). It illustrates the typically fast exchange of an unhindered equatorial hydrogen atom (H-2), the slow exchange of H-4 and H-6 which are syn-axial with O-2, and the intermediate rate of the axial H-1, H-3 and H-5 atoms. The ratio of exchange rates between H-4 and H-6 on the one hand, and H-2 on the other, would be sufficiently large to ensure selective deuteration if the other hydrogen atoms would not have intermediate rates. One could therefore expect selective deuteration in the case of neo-inositol (1,2,3/4,5,6-cyclohexanehexol) which has only equatorial and syn-axial hydrogen atoms; but, this being a rare compound of no practical importance, we did not carry out this experiment. None of the other inositols could be expected to show the required selectivity.

A methoxyl group provides additional steric hindrance; hence the inositol methyl ethers were investigated. It was found that a neighbouring methoxyl group retards the exchange of a hydrogen atom, the extent of the retardation depending on the steric relationship. For full details the original paper (ref. 22) should be consulted; suffice to say that particularly strong hindrance is exerted by an equatorial methoxyl group on the exchange of a neighbouring equatorial hydrogen atom. Such a methoxyl group provided us with our first example of selective deuteration: in L-2-O-methyl-chiro-inositol (4) (quebrachitol, a product found in many plants), after 15 minutes' reaction, ~90% of H-2 was replaced by deuterium but there

was little exchange in the other positions. A different specificity is achieved after 8 hours' reaction when H-4, H-5 and H-6 were almost completely replaced but none of the other hydrogen atoms had exchanged.

Our experiments with inositols, and also with cyclohexanepentols, allowed us to draw some conclusions as to the favoured geometrical arrangements of the exchange reaction. It occurs on the surface of the nickel catalyst, and the site of the molecule at which the substrate is preferentially adsorbed is the site at which the reaction will be fastest. For rapid exchange, a hydrogen atom must be geminal to a free hydroxyl group; these are, therefore, two points of attachment to the catalyst surface. But, as Balza and Perlin (ref. 20) have already noted, for rapid exchange another hydroxyl group is required; hydrogen atoms geminal to isolated hydroxyl groups exchange very slowly. The second hydroxyl group should, preferentially, be on a neighbouring carbon atom, gauche to both the hydrogen atom and the geminal hydroxyl group; the three hydrogen atoms then form a close-knit site for adsorption. An equatorial hydroxyl group on the neighbouring carbon atom is, therefore, more effective in promoting exchange than an axial one. A neighbouring methoxyl group does not function as a site of adsorption.

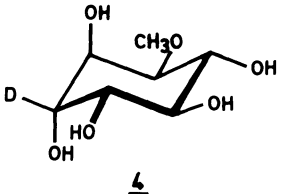
The protium-deuterium exchange over Raney nickel is accompanied by two other reactions: epimerization and deoxygenation. Fortunately they are both much slower than the exchange and, after the short runs used by us for selective exchange, only very small signals of other compounds were apparent in the n.m.r. spectra. It is, nevertheless, imperative in the preparation of labelled compounds by this method to recrystallize (or otherwise purify) the product.

Methyl glycosides

Free sugars cannot be deuterated by the Koch-Stuart method as they are reduced to alditols by Raney nickel; derivatives have to be used in which the reducing group is protected. We have only considered derivatives which can be readily prepared in one (or at the most, in two) steps from the sugars or are commercially available, and from which the sugar can be regenerated in high yield by a simple procedure. Obviously, derivatives could be tailor-made so as to provide good selectivity in deuteration; but with that effort, one can just as well use the traditional chemical procedures. We have studied methyl glycosides and anhydro-sugars as suitable derivatives.

One example of selective deuteration has already been described: Barker and co-workers (ref. 21) found that in methyl α-D-threofuranoside H-3 was exchanged to the extent of nearly 85%, with little exchange of the other hydrogen atoms.

In the methyl hexopyranosides, the H-6's exchange slowly, there being no neighbouring hydroxyl groups. The rate of exchange in the other positions depends on the configuration, and is also affected by the presence of the methoxyl group. Its effect, in the anomeric position, is different from that of the methyl ether group in cyclitols: it hinders more when equatorial rather than axial, and more when cis rather than trans to H-2. Thus, the exchange of H-2 in methyl β-D-glucopyranoside is very slow; in methyl α-D-glucopyranoside, on the other hand, it is exchanged rapidly.



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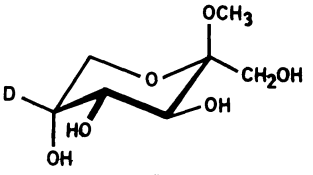

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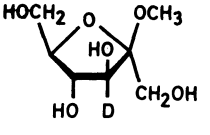

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It seemed to us that methyl α-D-galactopyranoside would provide a good example of selective deuteration: H-3 is syn-axial with O-1, and H-2 with O-4; one would expect that only H-4 would react rapidly. The exchange, however, was not as selective as hoped for (Table 1); although H-4 does exchange rapidly, there is also considerable exchange of H-2. The syn-axial hydroxyl group (on C-4) does not retard the reaction as much as the syn-axial methoxyl group (on C-1). The homomorphous ketoside, methyl β-D-fructopyranoside (5), however, showed satisfactory selectivity: only H-5 is exchanged rapidly. (The formulae indicate the position of deuteration.) In this case, H-3 is hindered also by the neighbouring hydroxymethyl group. In this way, D-fructose labelled on C-5 can be readily prepared. In methyl β-D-galactopyranoside, H-2 is hindered both by the methoxyl group and by O-4; hence this compound can be labelled at C-3 and C-4 without substantial deuteration in

TABLE 1. Hydrogen (%) remaining in various positions of some carbohydrates after exchange with deuterium

Compound	T (°)	t (min)	H-1	H-2	H-3	H-4	H-5	H-6_R	H-6_S
L-2-O-Methyl-*chiro*-inositol	90-95	15	100	100	100	95	90	∿10	
Methyl α-D-galactopyranoside	85	25	100	75	100	15	100	100	100
Methyl β-D-galactopyranoside	100	10	100	92	14	8	100	100	100
Methyl β-D-glucopyranoside	100	12	100	87	0	46	100	74	90
Methyl β-D-fructopyranoside	100	60	>96		>96	>96	0	100	100
Methyl β-D-fructofuranoside	80	40	>84		5	88	100	>84	92
1,6-Anhydro-β-D-galactopyranose	85	5	100	93	7	94	100	100	100
1,6-Anhydro-β-D-mannopyranose	100	360	100	93	18	90	100	100	100
1,6-Anhydro-α-D-galactofuranose	100	360	100	0	84	100	>97	100	100

the other positions. None of the other pyranosides we studied yielded satisfactory results (ref. 23).

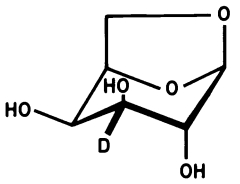
The deuterium exchange of the methyl pentofuranosides has already been studied by Barker and co-workers (ref. 21). The rate of exchange is controlled mainly by the methoxyl group: H-2 and H-3 exchange slowly if they are *cis* to the methoxyl group. Thus, in methyl α-D-xylofuranoside H-2, in methyl β-D-xylofuranoside H-3, is exchanged most rapidly. However, in contrast to the pyranosides, the hydrogen atoms on C-5 also exchange readily. When both H-2 and H-3 are *cis* to the methoxyl group, as in methyl α-D-lyxofuranoside and methyl β-D-ribofuranoside, one of the H-5 hydrogen atoms exchanges more rapidly than any other one. The methyl aldohexofuranosides can not be labelled selectively because the hydrogen atoms on C-5 and C-6 exchange readily.

Amongst the furanosides there was found only one instance of successful labelling: methyl β-D-fructofuranoside (6) can be deuterated on C-3 (Table 1).

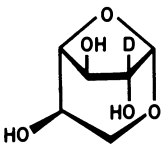
Anhydro-sugars

1,6-Anhydroaldohexoses offer a good opportunity for selective deuteration because their bicyclic rigid structure presents more steric hindrance, and they contain fewer free hydroxyl groups than the glycosides. Several 1,6-anhydro-sugars are readily prepared, and these were the only ones that we investigated. All the anhydro-sugars regenerate the parent sugar on acid hydrolysis.

The 1,6-anhydrohexopyranoses behave, in respect of the exchange reaction, like the methyl pyranosides but the anhydro bridge presents additional steric hindrance; if H-3 is axial, it is not exchanged. An equatorial H-2 is also severely hindered by the bridge. Hence we found that 1,6-anhydro-β-D-galactopyranose (7) is readily labelled on C-3 (Table 1); H-4 is *syn*-axial with O-2 and lacks a *gauche* neighbouring hydroxyl group, and H-2, in addition to being *cis* to O-1, also lacks the *gauche* hydroxyl group. 1,6-Anhydro-β-D-mannopyranose is also selectively labelled, though less satisfactorily, on C-3. Both anhydrides are easily prepared (ref. 24). 1,6-Anhydro-β-D-altropyranose can readily be labelled on C-4, a reaction of little interest as altrose has no biological importance.



7



8

The anhydrofuranoses used to be derivatives prepared with considerable difficulty in small yield; a recent method (ref. 25), however, allows the preparation of four of the isomers by a one-pot procedure in reasonable yield. Only these four diasteromers were submitted to deuteration. When H-2 and H-3 are *exo* (*manno*-isomer), they are both exchanged rapidly; when they are *endo* (*allo*, *talo*), they are exchanged slowly. On the other hand, when H-5 is *endo*, it is exchanged fairly rapidly but when it is *exo*, it is very resistant to exchange. Hence, in 1,6-anhydro-α-D-galactofuranose (8). with H-2 and H-5 *exo* and H-3 *endo*, only H-2 exchanges rapidly (Table 1), providing another example of selective deuteration. It is interesting that in 1,6-anhydro-α-D-talofuranose (H-2 and H-3 *endo*, H-5 *exo*), all of the hydrogen atoms resist exchange.

Summary

This research, conducted in collaboration with Dr. L. Odier in Grenoble, unearthed several examples of selective deuteration by the Koch-Stuart procedure over a short period of time.

Galactose can be labelled by deuterium on C-3 (via the 1,6-anhydropyranose), on C-2 (via the 1,6-anhydrofuranose), and on C-3 and C-4 (via the β-pyranoside); fructose on C-5 (via the β-pyranoside) or on C-3 (via the β-furanoside); and mannose on C-3 (via the 1,6-anhydropyranose). We have found no derivative which allows the selective deuteration of glucose.

It should be emphasized that the extent of deuterium exchange by this method is not always reproducible. Because the catalyst is stored under a solvent (deuterium oxide), it can not be weighed accurately and its amount may vary from one run to another; its activity is affected by age; and, as the reaction is heterogeneous, its rate will depend on the method and rate of stirring which is not rigorously controlled. It is recommended that, to achieve a planned extent of deuterium substitution, a run be made over a shorter time than shown in Table 1, an aliquot be tested by filtering off the catalyst and recording a ^{1}H or a ^{13}C-n.m.r. spectrum; and, according to the results thus obtained, the deuteration be continued.

It is not possible to achieve complete selectivity of labelling by exchange over Raney nickel (unless the compound has only one exchangeable hydrogen atom). In most cases this will not matter. Labelling by deuterium is being used extensively for the assignment of n.m.r. spectra, for the study of reaction pathways and mechanisms, biosynthesis and metabolism. For most of these uses, the essential feature is the ability to recognize a particular hydrogen or carbon atom; for this, it is not imperative that the labelling by deuterium be complete, nor does it matter if there are small amounts of deuterium in other positions. In fact, incomplete deuteration may be an advantage. One could, for example, think of a case where D-glucose, labelled as the methyl β-D-pyranoside (Table 1) could be useful; the hydrogen on C-3 had been completely replaced but only half of that on C-4. Both positions are therefore labelled but differently; and both can be recognized in the n.m.r. spectra.

Even if deuteration is not completely specific, the simplicity and rapidity of this method of deuteration warrants it use: stereospecific deuteration by chemical reactions is usually a long, multi-step procedure. Moreover, the method is cheap as it uses only deuterium oxide, which is the most economical source of deuterium.

If tritiated water is used instead of deuterium oxide, tritium-labelled sugars are obtained. The ratio of exchange rates is less critical now because complete exchange is not attempted: only a minute amount of tritium is introduced. Thus methyl α-D-galactopyranoside — not quite suitable for selective deuteration — can be labelled by tritium; the ^{3}H-n.m.r. spectrum of the product shows only one signal, corresponding to H-4 in the ^{1}H-n.m.r. spectrum.

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