Carbohydrates in Solution: Studies with Stable Isotopes

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Received October 4, 1985 (Revised Manuscript Received May 5, 1986)

Carbohydrates are polyhydroxy aldehydes and ketones that form essential structural elements in nature, confer individuality to biological surfaces, and are vital intermediates in the production and storage of energy in biological systems. They also provide a complete set of diastereomers for the study of structure–function relationships in chemistry and biochemistry, and many exhibit complex solution behavior. Monosaccharides, for example, exist in solution as interconverting linear and cyclic forms,[1,2] additionally complicated by the potential of each to adopt various conformations.[3,4] Chemical and biological reactions of these "simple" molecules often involve only one of the equilibrating forms,[5] and mechanistic interpretations must take account of the dynamics of tautomer interconversion.

Cyclic forms of monosaccharides occur as glycosides in oligo- and polysaccharides, glycoproteins and glycolipids, nucleotides, and polynucleotides. In all, conformation is a critical determinant of biological behavior.

For the past 10 years, we have been studying the solution conformations of oligosaccharides of the type 2 blood group, using synthetic oligosaccharides enriched at specific sites with stable isotopes, particularly ^{13}C. We reasoned that ^{13}C enrichment would facilitate the observation of interresidue ^{13}C–^{13}C and ^{13}C–^{1}H couplings, providing a tool for elucidating O-glycosidic bond conformations. This assumption proved correct but, in addition, we found that stable isotope enrichment combined with NMR spectroscopy (^{1}H, ^{13}C, ^{2}H) and mass spectrometry provided opportunities to monitor carbohydrate reactions in solution and to elucidate their mechanisms.

In ^{13}C-enriched compounds, ^{13}C–^{13}C coupling to the enriched carbon permits unequivocal assignment of nearest-neighbor carbons (Figure 1). Our early studies led to the reassignment of monosaccharide resonances,[6] a process that continues as new compounds are prepared.[7]

Carbon resonances can be assigned also by irradiating individual proton resonances with weak coherent radiation while observing the carbon spectrum[8] or by using two-dimensional heteronuclear shift correlation spectroscopy.[8a,9]

^{13}C enrichment allows NMR measurements of enriched site(s) to be made up to 10^3 times more quickly and with higher precision than with unenriched materials. ^{2}H enrichment allows the fate of specific hydrogens in a reaction to be determined by ^{1}H, ^{13}C, and/or ^{2}H NMR spectroscopy. Detection of deuterated carbons is hindered by ^{13}C–^{2}H coupling, loss of NOE, and increased relaxation times, but incorporation of ^{13}C at sites of deuteration can reduce these difficulties.

For conformational studies, three-bond (vicinal) couplings (^{3}J) can be related approximately to the torsion angles between the coupled nuclei.[10] Vicinal coupling between protons is commonly used, but ^{13}C–^{13}C[11] and ^{13}C–^{1}H[12] couplings are equally useful, and are easy to obtain from spectra of ^{13}C-enriched compounds (Figures 1 and 2). Approximate "Karplus" curves are known for CCCH, COCH, CCCC, and COCC coupling pathways in the carbohydrates.[13,14]

The Cyanohydrin Reaction

In the historically important cyanohydrin, or Kiliani, reaction,[15] cyanide condenses with an aldose (or ketose) under alkaline conditions to yield, after hydrolysis, a pair of epimeric carboxylate salts (aldonates). Fischer[16] extended the reaction by reducing the aldonic acid lactones (aldonolactones) with sodium amalgam to generate new reducing sugars. The Kiliani–Fischer

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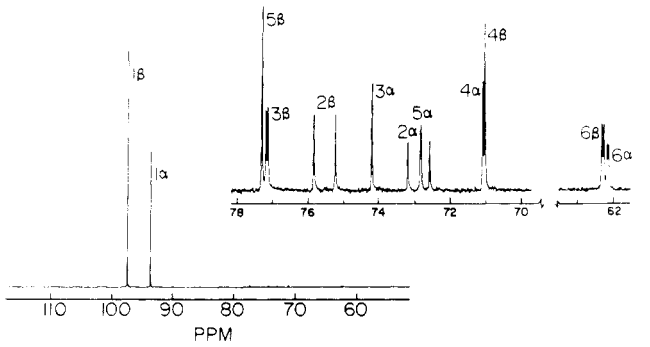

Figure 1. 75-MHz ^{1}H-decoupled ^{13}C NMR spectrum of D-[1-^{13}C]glucose (99 atom % ^{13}C) in ^2H_2O, showing the enriched and unenriched (expanded) regions. $^1J_{CC}$ can be used to identify unequivocally the C2 carbons of the α- and β-pyranoses ($^1J_{C1,C2}{}^{\alpha}$ = 46.2 Hz, $^1J_{C1,C2}{}^{\beta}$ = 46.0 Hz). Other ^{13}C–^{13}C couplings can also be observed: $^2J_{C1,C5}{}^{\alpha}$ = 1.8 Hz, $^3J_{C1,C6}{}^{\alpha}$ = 3.3 Hz, $^2J_{C1,C3}{}^{\beta}$ = 4.5 Hz, and $^3J_{C1,C6}{}^{\beta}$ = 4.1 Hz. These two- and three-bond couplings can be used to assist in the assignment of resonances[7] and in conformational analysis.[6]

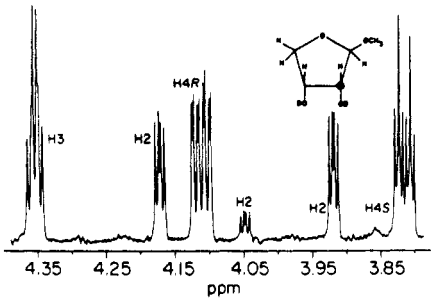

Figure 2. 600-MHz ^{1}H NMR spectra of the H2–H4 region of methyl β-D-[2-^{13}C]erythrofuranoside. Comparison with the spectrum obtained with the unenriched compound allows precise determinations of J_{CH}. The intraring ^{13}C–^{1}H couplings ($^2J_{C2,H1}$ = br, $^1J_{C2,H2}$ = 149.2 Hz, $^2J_{C2,H3}$ = 0 Hz, $^3J_{C2,H4R}$ = 1.6 Hz, $^3J_{C2,H4S}$ = 3.5 Hz) can be used in conjunction with J_{HH} and J_{CC} to evaluate furanose conformation and dynamics.[49]

synthesis is the traditional method of chain extension of carbohydrates.

Cyanohydrins (aldononitriles) formed in the Kiliani reaction are significantly more alkali-labile than the alkyl cyanohydrins which hydrolyze to carboxylate salts via their corresponding amides, suggesting different mechanisms. Indeed, Varma and French[17] proposed on the basis of chromatographic analyses that cyanohydrins cyclize to 1,5-imidolactones prior to amide formation and that cyclic carbinolamines are intermediates in the conversion of imidolactone to amide.

We decided to investigate further to determine if the observation of Kuhn and Klesse,[18] namely, that cyanohydrins formed in pyridine could be reduced catalytically in dilute acid to hexoses, might be extended to form and stabilize cyanohydrins in aqueous solution for reduction directly to aldoses. In addition, we wished to clarify the role of cyclic forms in cyanohydrin hydrolysis.

The Kiliani reaction is complicated by the formation of pairs of structures (epimers) of all intermediates, and by the lability of these intermediates. Nevertheless, we were able to conduct a detailed study of the condensation of K^{13}CN with D-erythrose leading to the formation of D-[1-^{13}C]arabinonate and D-[1-^{13}C]ribonate.[19]

(17) Varma, R.; French, D. *Carbohydr. Res.* **1972**, *25*, 71.
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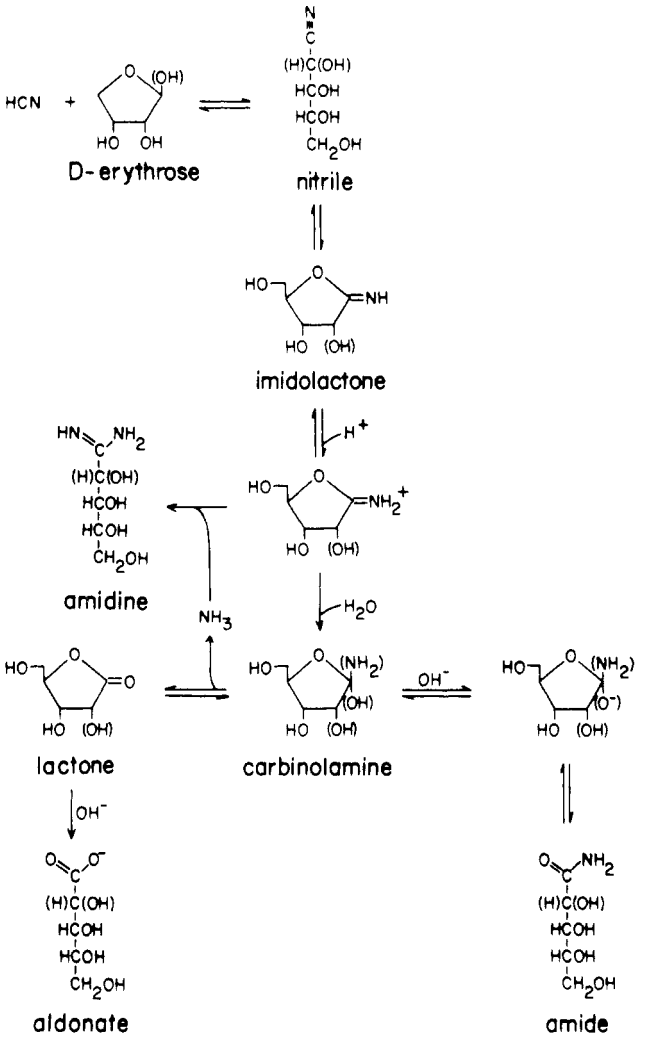
Scheme I



The ^{13}C NMR spectrum of a reaction mixture at pH 7.0 after 10 h shows resonances from twelve enriched carbons, eight of which were assigned by comparison with spectra of standard enriched compounds. By collecting "time-lapse" spectra, appearance and disappearance profiles of specific resonances were obtained as a function of pH, temperature, and reactant concentration. From these data, a general pathway was proposed (Scheme I), in which cyclic structures play a central role. Take, for example, the imidolactones.

Two resonances appear immediately following nitrile formation and before amide accumulation; at pH 10.5, they account for ~65% of the enriched carbon. Acidification of this mixture with HCl generates lactones, amides form in alkali,[20] and amidines form on addition of ammonium chloride. Below pH 9.5, amidine formation decreases, as expected for imidate aminolysis.[21]

"Imidolactones" formed by the addition of ^{13}CN to DL-[2-^{13}C]erythrose and DL-[3-^{13}C]erythrose were examined. Here two noncontiguous carbons can be monitored simultaneously and chemical shifts and ^{13}C–^{13}C couplings obtained without the spectral complexity

(19) (a) Serianni, A. S.; Nunez, H. A.; Barker, R. *J. Org. Chem.* **1980**, *45*, 3329. (b) A similar study on D-arabinose using [^{13}C]cyanide and [^{13}C,^{15}N] cyanide has been conducted by Blazer, R. M.; Whaley, T. W. *J. Am. Chem. Soc.* **1980**, *102*, 5082.
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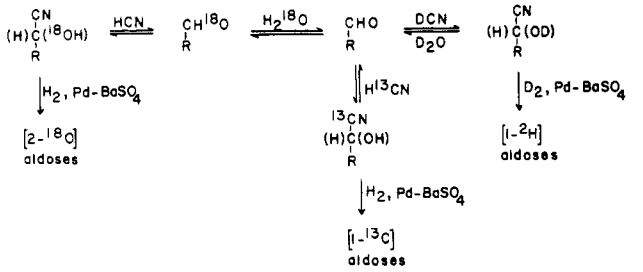
(21) Hand, E. S.; Jencks, W. P. *J. Am. Chem. Soc.* **1962**, *84*, 3505.

Scheme II



that arises from one-bond ^{13}C–^{13}C couplings. All resonances corresponded closely to those of structurally related 1,4-lactones, suggesting that imidolactones have five-membered rings. Further support for the formation of 1,4-imidolactones came from studies with 5-deoxypentononitriles, which cannot form six-membered rings yet hydrolyze at essentially the same rates as those that can.

The Kiliani reaction illustrates the complications often encountered in carbohydrate reactions due to ring formation. It provided a convincing example of the value of site-specific ^{13}C enrichment in the elucidation of a complex reaction pathway, and, on a practical level, led to the development of simple and general methods for incorporating isotopes of carbon, hydrogen, and/or oxygen into carbohydrates.[22]

Synthesis of Labeled Carbohydrates

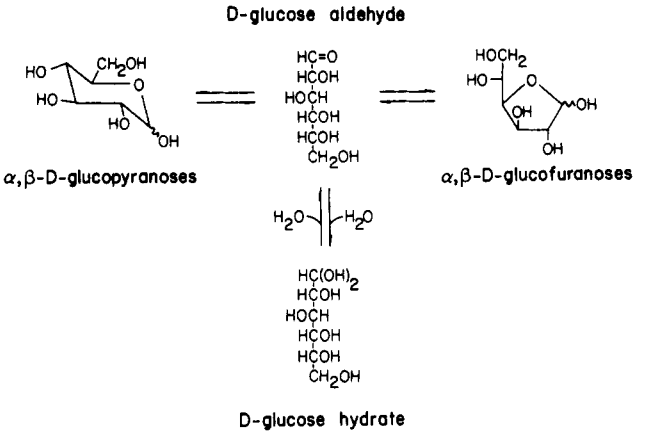
Aldoses condense with cyanide at pH 7.5–8.5 to form epimeric aldononitriles almost quantitatively. At pH 4.5 these nitriles are stable, and the reversal of cyanide addition and hydrolysis of cyanohydrins are negligible, permitting reduction to aldoses in good yield.[23] This simple synthetic sequence (Scheme II) has been used to prepare a wide range of monosaccharides enriched with isotopes of carbon, hydrogen, and/or oxygen.[24] Enriched compounds can be modified by chemical and/or enzymic methods; for example, DL-[1-^{2}H]-glyceraldehyde (prepared by reduction of DL-glyceronitrile with $Pd/BaSO_4$ and $^2H_2$) can be converted to D-[4-^{2}H]glucose and D-[4-^{2}H]fructose using commercially available enzymes and substrates. Both compounds are difficult to prepare by chemical methods alone.

The Anomerization Reaction

Spontaneous intramolecular ring-opening and ring-closing (anomerization) reactions are characteristic of reducing sugars. Solutions may contain furanose and/or pyranose rings and a carbonyl form and its hydrate (*gem*-diol) (Scheme III).[1,2] The potential for the presence of many conformers of each is worth stressing.

Most studies of anomerization have used crystalline anomers to measure overall rates of interconversion between cyclic forms (see, however, ref 25) under non-

Scheme III



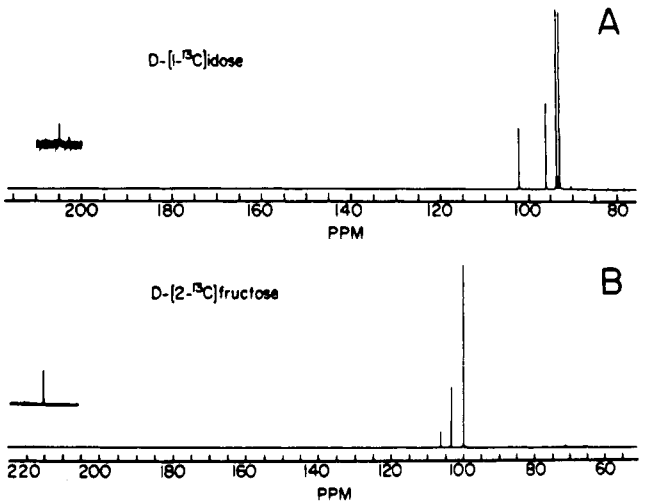


Figure 3. 75-MHz ^{1}H-decoupled ^{13}C NMR spectra (~25 °C) of D-[1-^{13}C]idose[8a] (A) and D-[2-^{13}C]fructose (B) in ^2H_2O, showing only the enriched carbons (99 atom % ^{13}C). ^{13}C chemical shifts (in ppm): D-idose (α-furanose, 103.1; β-furanose, 96.8; α-pyranose, 94.4; β-pyranose, 93.7; hydrate, 91.2; aldehyde, 205.7); D-fructose (α-furanose, 105.9; β-furanose, 103.0; β-pyranose, 99.5; ketone, 214.9).

equilibrium conditions.[26] Overall rate constants are composites of the more fundamental unidirectional rate constants and cannot be interpreted in mechanistic terms, and many sugars cannot be studied for lack of crystalline anomers.

The two-site saturation-transfer method of Forsén and Hoffman[27] can be applied, however, to anomerizing systems at equilibrium.[8a,28,29] For example, if nucleus A (e.g. carbonyl form) is "labeled" by applying saturating radiation at its resonance frequency and experiences chemical exchange to become nucleus B (e.g. β-furanose form) while saturated, it will not contribute to the resonance intensity of B. As saturation times are increased, the resonance intensity of B decays until a new equilibrium intensity obtains. This new intensity is determined by how fast B relaxes (T_1 of site B) and how fast B converts to A (one rate constant for the interconversion). These two rate constants can be re-

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(25) Anderson and co-workers have used gas-chromatographic data and a computer modeling program to predict unidirectional rate constants for D-galactose anomerization (Wertz, P. W.; Garver, J. C.; Anderson, L. *J. Am. Chem. Soc.* **1981**, *103*, 3916).

(26) For classical reviews, see: Pigman, W.; Isbell, H. S. *Adv. Carbohydr. Chem. Biochem.* **1968**, *23*, 11. Isbell, H. S.; Pigman, W. *Ibid.* **1969**, *24*, 13.

(27) Forsén, S.; Hoffman, R. A. *J. Chem. Phys.* **1963**, *39*, 2892.

(28) Serianni, A. S.; Pierce, J.; Huang, S.-G.; Barker, R. *J. Am. Chem. Soc.* **1982**, *104*, 4037.

(29) Pierce, J.; Serianni, A. S.; Barker, R. *J. Am. Chem. Soc.* **1985**, *107*, 2448.

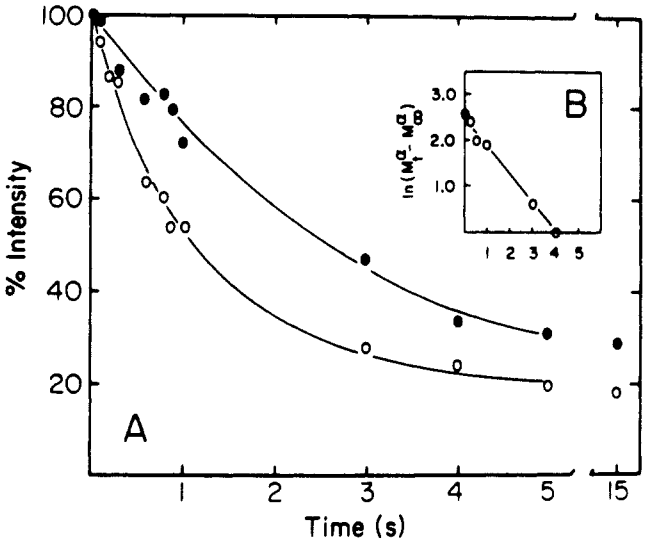

Figure 4. ^{13}C saturation-transfer experiment on D-[1-^{13}C]-erythrose (0.1 M) in ^2H_2O (p^2H 5.0) and acetate buffer (50 mM) at 55 °C. (A) Plot of resonance intensity vs. saturation times, showing different rates of decay for the α-furanose (open circles) and β-furanose (closed circles). (B) Semilogarithmic plot of the data in A for the α-furanose, from which a ring-opening rate constant of 0.40 s^{-1} is obtained. Similar treatment for the β-furanose yields a value of 0.19 s^{-1}. Under these conditions, the effect of saturation on the intensity of the hydrate signal is small, and only an upper limit for the rate of dehydration (<0.05 s^{-1}) is obtained.

solved experimentally. The technique is limited to rate constants in the approximate range 0.05–10 s^{-1},[30] but values in the range 10–200 s^{-1} can be obtained by line-broadening measurements as described by Gutowsky and Holm.[31]

Before anomerization can be studied in equilibrated solutions, the composition of these solutions must be established.[1,2] For aldoses, ^1H NMR observation of the hemiacetal protons can be used, while for ketoses, ^{13}C NMR of ^{13}C-enriched forms is advantageous (Figure 3). In aldoses, comparisons of ^1H-coupled and -decoupled ^{13}C spectra permit the assignment of resonances to specific forms, since one-bond ^{13}C–^1H coupling varies with ring size and anomeric configuration.[8a,32] When differential relaxation and NOE effects are minimized, quantitative analysis is straightforward. With ^{13}C enrichment, carbonyl forms can be quantified in amounts less than 0.1 mol % (Figure 3),[8a,28,29,33] permitting equilibrium compositions, equilibrium constants, and standard thermodynamic parameters ($\Delta G°$, $\Delta H°$, $\Delta S°$) to be obtained.[28]

Saturation-transfer methods were applied first to the tetroses[28] whose aqueous solutions contain approximately 88% furanose, 10% hydrate, and 2% carbonyl forms. Unidirectional rate constants for ring-opening of the α- and β-tetrofuranoses were determined by observing the effect of saturating either H1 or C1 of the aldehyde on the resonance intensities of the cyclic forms (Figure 4). Approximate ring-closing rate constants were calculated from these ring-opening rate constants and equilbrium constants (Table I). Temperature effects on rate constants gave activation parameters ($\Delta G^‡$, $\Delta H^‡$, $\Delta S^‡$) for each process.[28]

Table I
Unidirectional Rate Constants[a] for the Anomerization of D-Tetroses in ^2H_2O[b]

T, ±1 °C	NMR	form	k_{open} ± 10%	k_{close} ± 18%
51	^1H	α-threo	0.057	1.2
		β-threo	0.19	3.2
51	^1H	α-erythro	0.25	
		β-erythro	0.14	
55	^{13}C	α-threo	0.11	2.2
		β-threo	0.36	5.3
55	^{13}C	α-erythro	0.40	
		β-erythro	0.19	

[a] Units of s^{-1}. [b] 0.1 M aldose in 50 mM sodium acetate in ^2H_2O, p^2H 5.0.

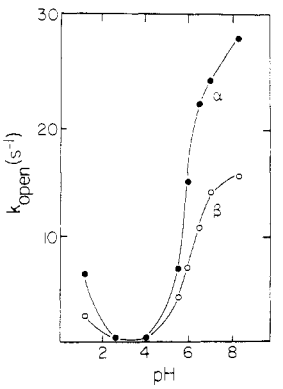

Figure 5. pH dependence of the ring-opening rate constants for D-[1-^{13}C]ribose 5-phosphate (0.3 M sugar in 15% ^2H_2O at 24 °C), showing the difference in behavior between α-furanose (closed circles) and β-furanose (open circles). Rate constants were obtained from line-broadening experiments except for the values at pH 2.3 and 4.0, which were obtained from saturation-transfer experiments.

The carbonyl form appears to be the only intermediate in the interconversion of furanoses. When both the aldehyde and one of the hemiacetal carbon signals were saturated simultaneously,[28] the intensities of the remaining hemiacetal carbon signals were not further decreased. If a second intermediate was involved, application of the second saturating frequency should have reduced the remaining resonance(s).

Ring-opening rates decrease through the series α-erythrose > β-threose > β-erythrose > α-threose in parallel with the number of 1,2-cis interactions between hydroxyl groups. Rates of dehydration (hydrate to aldehyde) are considerably slower than ring-opening and -closing rates. Earlier, a "pseudoacyclic" intermediate had been proposed to explain the slow rate of ^{18}O exchange into ring forms from solvent water.[26,34] The slow rate of dehydration, however, accounts for this observation.

Although hexoses (glucose, mannose, fructose) are more common, we chose next to study the biologically important aldose and ketose phosphates.[29] Phosphorylation increases anomerization rates[35] consistent with

(30) Campbell, I. D.; Dobson, C. M.; Ratcliffe, R. G.; Williams, R. J. P. *J. Magn. Reson.* **1978**, *29*, 397.
(31) (a) Gutowsky, H. S.; Holm, C. H. *J. Chem. Phys.* **1956**, *25*, 1228. (b) Anet, F. A. L.; Basus, V. J.; *J. Magn. Reson.* **1978**, *32*, 339.
(32) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293.
(33) The detection and quantification of the carbonyl and other minor forms by NMR spectroscopy is hindered by the dynamic range problem inherent to the method. The use of block-averaging and double-precision data minimizes this difficulty; recent hardware modifications offer further improvement [Davies, S.; Bauer, C.; Barker, P.; Freeman, R. *J. Magn. Reson.* **1985**, *64*, 155.] Nevertheless, we have detected the linear aldehyde form of D-[1-^{13}C]ribose by ^{13}C NMR and estimate that it comprises ~ 0.03% of the total forms in aqueous solution at ~30°.
(34) Maier, G. D.; Kusiak, J. W.; Bailey, J. M. *Carbohydr. Res.* **1977**, *53*, 1.

their well-known sensitivity to acid–base catalysis. Rate enhancement increases with ionization of the phosphate group, the dianion being the better catalyst (Figure 5). The reaction at pH 4.2 is essentially H_2O-catalyzed, whereas at pH 7.5 both general (phosphate dianion) and specific (OH^-) base catalysis occurs. Catalysis depends also on the orientation of the phosphate group with respect to the anomeric center, and on ring configuration. In the pentose 5-phosphate series, ring-opening is faster in α-anomers than in β-anomers at both pH 4.2 and 7.0. In α-anomers, the anomeric hydroxyl and the phosphate group are trans, suggesting that the phosphate group does not act directly to deprotonate OH-1. Phosphate-facilitated protonation of the ring oxygen may explain the observed effects, with the phosphate group acting either directly or through a water molecule.

In several 2-ketohexoses,[29,36] the "α-furanose rule" is violated; in these structures β-anomers open faster. Apparently, proper phosphate orientation is prevented when the C1-hydroxymethyl group is cis to the C6-phosphate group. In addition, a phosphate group is more effective at C1 than at C6 in promoting ring-opening of β-anomers as shown by the ring-opening rate constants at 40 °C and pH 7.5 for 6-*O*-methyl-D-fructose, D-fructose 6-phosphate, and 6-*O*-methyl-D-fructose 1-phosphate, which are 1.3, 21, and 130 s^{-1}, respectively.

Anomerization is a good example of the intramolecular reactions that are important in organic chemistry and govern much of carbohydrate chemistry. Early studies[37-39] of acid-catalyzed ring-closure reactions (1,4-anhydride and furanoside formation) showed that ring configuration affects closure rates, with *ribo* > *xylo* > *arabino* > *lyxo*. This trend was explained by suggesting that when OH-2 eclipsed the leaving group, the transition state was destabilized, a situation that cannot arise during anomerization, since there is no leaving group. Despite the differences, it is clear that understanding ring-closing and -opening is essential to understanding carbohydrate reactivity.

Molybdate-Catalyzed Aldose C2 Epimerization

Epimerization, the inversion of a single chiral site, is important in the chemical and biochemical conversions of carbohydrates. Generally, it involves formation of a keto intermediate, which is converted by isomerization[40] or reduction to the desired epimer.

Recently, Bílik and co-workers[41,42] described a novel reaction in which C2 epimerization of aldoses is promoted by molybdate ion, apparently without the participation of 2-keto intermediates. A thermodynamic equilibrium of C2 epimers is formed with very small amounts of other isomers. Studies with tritiated aldoses were interpreted as showing that the reaction took place by the simultaneous exchange of H1 and H2.[42]

This interesting reaction offered the possibility of converting relatively undesirable [1-^{13}C]aldoses, such as D-[1-^{13}C]mannose, to their more useful [1-^{13}C]-enriched C2 epimers. The molybdate reaction, when applied to D-[1-^{13}C]mannose, yielded ~65% D-*gluco* and ~35% D-*manno* isomers, as predicted by Angyal[1] from pyranose interaction energies. However, instead of the expected [1-^{13}C]glucose, D-[2-^{13}C]glucose was produced[43] (Figure 6). Clearly the mechanism suggested by Bílik[42] is incorrect; epimerization involves carbon–carbon bond cleavage between C2 and C3 of the aldose.

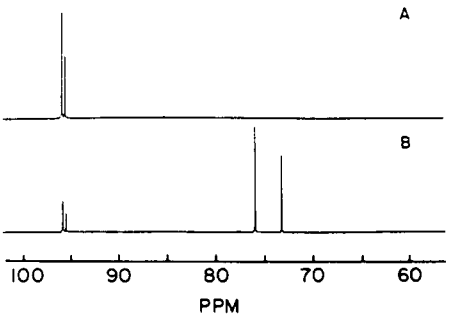

Figure 6. 75-MHz ^{1}H-decoupled ^{13}C NMR spectra of D-[1-^{13}C]mannose (A) and the reaction mixture resulting after treatment of D-[1-^{13}C]mannose (0.1 M) with molybdic acid (85%, 5 mM) at 90 °C for 4 h. Only enriched carbons are shown. The new resonances in B at 72.9 and 75.6 ppm are due to D-[2-^{13}C]-glucose generated during the reaction.

This conclusion was confirmed by using D-[1-^{13}C,^{2}H]mannose, which gave D-[2-^{13}C,^{2}H]glucose, consistent with Bílik's observation that H1 and H2 exchange during the reaction, but showing that it is accompanied by carbon exchange. D-[1,3-$^{13}C_2$,3-^{2}H]-Mannose yielded D-[2,3-$^{13}C_2$,3-^{2}H]glucose, demonstrating that the rearrangement occurs without the abstraction of H3 and without C3 transposition, eliminating mechanisms involving carbanion formation at C3 with subsequent attack at C1.

Further studies with structural analogues showed that O1, O2, and O3 are required for reactivity, that O4 facilitates the reaction but can be replaced by O5, and that stereochemistry affects reaction rates.[43]

On the basis of the effects of pH and temperature on reaction rates, molybdate self-oligomerization,[44] molybdate-sugar complexation, and aldehyde concentration, we postulate that the aldehyde, although least abundant, binds to dimolybdate in a complex that involves oxygen atoms at C1, C2, C3, and C4.[43]

On a practical level, molybdate-catalyzed C2 epimerization provides a simple synthetic tool for the synthesis of carbohydrates enriched with carbon, hydrogen, and/or oxygen isotopes.[24]

Enzyme Studies: D-Ribulose-1,5-bisphosphate Carboxylase/Oxygenase

Spinach-leaf D-ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase catalyzes the addition of CO_2 to RuBP (D-*erythro*-pentulose 1,5-bisphosphate) to generate two molecules of D-glycerate 3-phosphate.[45] It also catalyzes a second reaction, the addition of O_2 to RuBP, to generate one D-glycerate 3-phosphate molecule and one glycolate phosphate.[46]

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Scheme IV

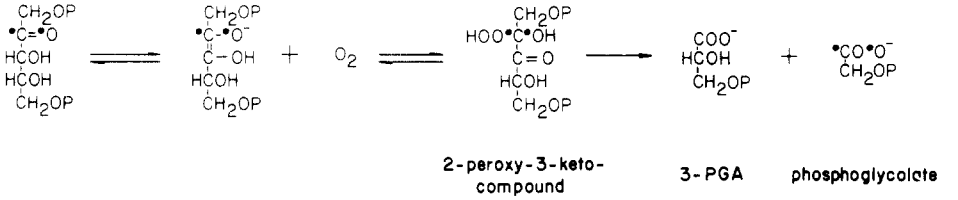
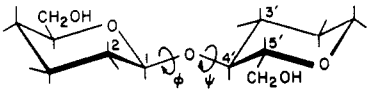


Chart I



Although the CO_2-fixing mechanism had been supported experimentally,[45] the position of RuBP cleavage in the oxygenase reaction had not been studied. D-[2-^{13}C]RuBP, prepared from D-[2-^{13}C]ribose 5-phosphate by using D-ribose-5P-isomerase, phosphoribulokinase, and Mg^{2+}ATP,[47] was incubated with $^{13}CO_2$ and the product D-glycerate 3-phosphate analyzed by GC–MS. One-half of the glycerate contained ^{13}C at both C1 and C2, while the other half was unenriched, confirming that, within the limits of the analysis, the carboxylase reaction involves cleavage of the C2–C3 bond of RuBP.[48]

In the oxygenase reaction, all of the ^{13}C from D-[2-^{13}C]RuBP was found at the carboxyl group of glycolate phosphate, as was ^{18}O from D-[2-^{18}O]RuBP. These results demonstrate that cleavage occurs between C2 and C3 (Scheme IV) and that carbonyl oxygen exchange is not an obligatory step in the reaction.[48]

These experiments with stable isotopes demonstrate the mode of action of the enzyme simply and unequivocally. Radioisotopic methods would have required much more extensive development of degradative procedures to locate radiolabeled site(s) in the products.

Oligosaccharide Conformation

In oligo- and polysaccharides, once the sequence of residues, ring size, and linkages are known, the conformations of glycosyl rings, glycosidic bonds, and exocyclic fragments can be evaluated.

Pyranosyl residues in oligo- and polysaccharides generally assume conformations similar to those in simple glycosides; this is not unexpected in light of the substantial energy differences (2–4 kcal/mol) between the stable conformer(s) and the next higher energy conformations. In contrast, furanosyl rings exist in several rapidly interconverting conformations of comparable energy;[49a] in complex molecules (e.g., DNA, RNA), their conformations may be affected significantly by overall structure.

The conformation of an O-glycosidic bond is specified by two dihedral angles, ϕ and ψ (Chart I), and each can be evaluated by NMR. For example, ϕ can be estimated from coupling between C2 and C4′, or H1 and C4′, whereas ψ can be estimated from coupling between C1 and H4′, C3′, and/or C5′; these couplings can be measured with high precision with ^{13}C-enriched compounds. Conformation can also be deduced from the distances between pairs of protons obtainable from T_1s and/or spin-saturation or inversion effects on proton resonance intensities.[50] The latter approach is simplified by use of ^{2}H-enriched compounds.[50b]

Gagnaire and co-workers introduced ^{13}C enrichment to assess glycoside conformation using ^{13}C–^{13}C and ^{13}C–^{1}H coupling across the glycosidic bonds of several ^{13}C-enriched acetylated disaccharides.[51a,b] Perlin and co-workers[51c] used unenriched disaccharides and proton-coupled ^{13}C NMR with a resolution of ~2.0 Hz to obtain some interresidue couplings.

Our approach to the preparation of isotopically enriched oligosaccharides utilizes chemically prepared nucleoside diphosphate sugars containing enriched monosaccharides and highly specific glycosyltransferases isolated from various biological sources to form glycosidic bonds.

Conformational assessment of a type 2 blood group oligosaccharide is illustrated with the core disaccharide, methyl β-D-lactoside.[52] Several lactosides were prepared with single or multiple ^{13}C enrichment at C1, C2, and/or C3 of the Gal moiety and at C1′, C3′, and/or C4′ of the Glc moiety so that several intraresidue and all interresidue ^{13}C–^{13}C and ^{13}C–^{1}H couplings could be measured. Three-bond couplings in the Gal and Glc residues were similar to those found in respective methyl glycosides, indicating that the monosaccharide moieties retained their usual conformations.

Studies of T_1 values of ^{13}C nuclei in both residues suggest that lactoside tumbles somewhat anisotropically[53] and that rotation about the glycosidic bond is slow or absent. From this and various interresidue couplings, we concluded that the glycosidic bond has a stable conformation with $\phi \simeq 40°$ and $\psi \simeq 15°$. Couplings corresponding to the same dihedral angle are internally consistent; for example, those related to ϕ suggest dihedral angles between C1–C3′ $\simeq 105°$, C1–C5′ $\simeq 135°$, and C1–H4′ $\simeq 15°$. This conformation is very similar to that computed by Lemieux and co-workers from hard-sphere models and the exoanomeric effect.[50a,54]

The observation of substantially different couplings between atoms sharing a common central bond ($^3J_{C1,C3'}$ = 0 Hz, $^3J_{C1,C5'}$ = 1.6 Hz, and $^3J_{C1,H4'}$ = 4.9 Hz) is consistent with the conclusion that rotation about the

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glycoside bond is slow or absent. If rotation was rapid, both ^{13}C–^{13}C couplings would be more similar, representing an averaged value over a rotational itinerary.

Strategies similar to these have been extended to tri- and tetrasaccharides.[55,56] The question of what effect the addition of new residues has on the conformations of existing *O*-glycoside bonds in a growing oligosaccharide is beginning to be addressed; we have observed one case where measurable changes in orientation occur.[56] It would be interesting to extend these studies to complexes of these oligosaccharides with receptor proteins through which they display their biological functions. Future studies will focus on questions related to solution conformations, further exploration of the effects of configuration and substitution on structure, and extensions to other complex systems, including oligo- and polynucleotides.

Concluding Remarks

This Account describes several studies that illustrate the value of stable isotopes as probes of chemical and biochemical reaction mechanisms and of the structures of complex biomolecules. While these studies focus on carbohydrates, the limiting factor in all studies with stable isotopes is the availability of compounds enriched at specific sites. Our studies were made possible by the development by the Los Alamos National Laboratory of methods for producing ^{13}C of 99+% enrichment and our modification of the Kuhn method[18] to introduce isotopes of carbon, hydrogen, and/or oxygen at various sites in a wide range of monosaccharides. Advances in NMR spectroscopy and mass spectrometry, as well as improved chemical and biochemical synthetic methods, point to an increasing role for stable isotopes in studies of the structures and reactivities of complex molecules, especially those of biological origin.

We have not discussed the use of carbohydrates enriched with stable isotopes for metabolic studies where they can be applied with apparent safety in humans and the environment, areas in which radioisotopes must be applied with caution. Although radioisotopes are more easily detected, they provide virtually no information about the chemical or physical nature of their molecular environment; they can report little but their presence.

The ability to selectively monitor an enriched atom(s) in a molecule as its chemical or physical environment changes is an important feature of studies with stable isotopes. Unlike other reporter groups, stable isotopes do not significantly perturb physical or chemical properties (with, of course, the exception of isotope effects), so the information they provide more reliably reflects reality. In our view, a substantial initial investment in developing synthetic methods was repaid many-fold by the ease with which complex questions of mechanism and structure could be addressed. In other systems, similar investments can be expected to pay similar dividends.

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ESR Investigations of Molecular Cation Radicals in Neon Matrices at 4 K: Generation, Trapping, and Ion–Neutral Reactions

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Received April 22, 1986 (Revised Manuscript Received July 21, 1986)

The cations of most neutral parent molecules are radicals and hence amenable to ESR (electron spin resonance) study. However, their highly reactive nature, short lifetimes, and generation difficulties have been major obstacles to overcome in applying ESR and other spectroscopic methods to the major categories of cation radicals. Our experimental emphasis over the past several years has been to apply the rare gas (principally neon) matrix-isolation method to representative and fundamentally important types of cation radicals which cannot be readily investigated by other approaches.[1] For the smaller cations, the ESR assignment is more definitive and detailed comparisons with ab initio theoretical calculations are possible. The ability to generate, characterize, and manipulate molecular ions in matrices or during the condensation process has enabled the study of ion–neutral reaction types which have previously eluded spectroscopic monitoring. Numerous questions concerning the chemistry of the upper atmosphere, interstellar clouds, and high-energy environments could be probed by this ion–neutral reaction approach described in this Account.

Open-shell cations are especially difficult to study in the gas phase by high-resolution microwave spectroscopy or LMR (laser magnetic resonance), and nuclear

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