Solution Structures of the Keto Sugars and Their Biologically Important Phosphate Esters

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Carbohydrates, which have long been of interest to chemists as model compounds for the study of the stereochemical aspects of chemical reactions, have recently attracted the interest of biochemists who have examined the stereochemical aspects of their enzyme-catalyzed reactions. A given monosaccharide can exist in several forms in solution, including the acyclic free carbonyl and hydrated carbonyl (*gem*-diol) forms and the cyclic furanose and pyranose hemiacetal forms. A question arises, however, as to whether enzymes which utilize these sugars as substrates are able to *use* each of the tautomeric forms. For example, an enzyme may be able to bind all forms but utilize only one in the catalytic reaction. Where only a single form is utilized, the other forms either may be converted to the reactive form by the enzyme or may function as inhibitors. The latter case is especially significant if the reactive form is present in a very low proportion in the tautomeric equilibrium. It is also possible that a given enzyme might bind and utilize only one of the forms present in the equilibrium. It is conceivable that, if the substrate form were used at a faster rate than it was formed in the tautomeric equilibrium, the rate of the enzyme-catalyzed reaction would be under "tautomeric control".

In order to describe the kinetic mechanism of an enzyme whose substrate exists in multiple tautomeric forms in solution, it is necessary to determine the proportion of each form present in the equilibrium and the mode of interaction of each form with the enzyme. Enzymes which utilize phosphorylated esters of keto sugars have received much attention in this regard,[1-7] and it has been found that they do indeed recognize and differentially utilize tautomeric forms of their substrates.

It is the purpose of this Account to summarize the methods by which the tautomeric compositions of these substrates have been established. The present review will concern itself with the tautomeric compositions of the phosphorylated keto sugars D-fructose 1,6-bisphosphate, D-fructose 6-phosphate, and 1,3-dihydroxy-2-propanone phosphate, and the keto sugars D-fructose, D-tagatose, L-sorbose, and D-psicose. Although every chemist might not be interested in the structures of these molecules per se, the *methods* by which these structures have been established are of more general interest because of their applicability to the structural characterization of a variety of organic compounds.

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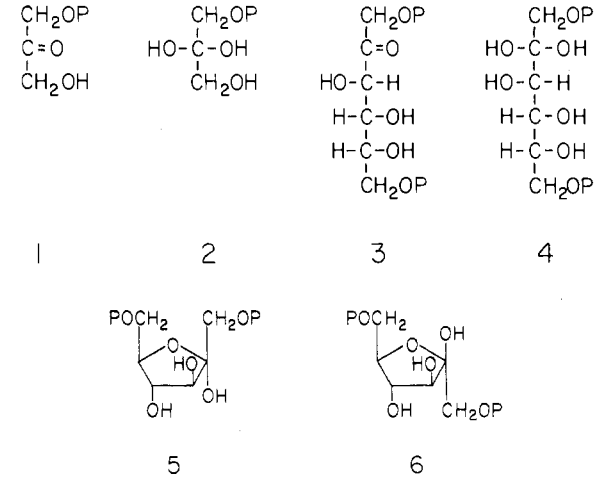
Characterization of Acyclic Forms

1,3-Dihydroxy-2-propanone phosphate can exist in the acyclic free keto (**1**) and hydraed keto (**2**) forms in solution, but D-fructose 1,6-bisphosphate, in addition to the free keto (**3**) and hydrated keto (**4**) forms, can also exist in the cyclic α-furanose (**5**) and β-furanose (**6**)



forms. In order to distinguish between cyclic and acyclic forms, Gray and Barker[8] examined analogs which could only exist in acyclic forms and compared their ir, NMR, and uv spectra (all obtained in D_2O) with those of analogs having the potential to exist in both cyclic and acyclic forms. The analogs used in these studies, in addition to 1,3-dihydroxy-2-propanone phosphate (**1**) and D-fructose 1,6-bisphosphate (**3**), were D-*glycero*-D-*altro*-octulose 1,8-bisphosphate (**7**), D-*erythro*-pentulose 1,5-bisphosphate (**8**), 5,6-dideoxy-D-*threo*-hexulose 1-phosphate (**9**), and 1,5-dihydroxy-2-pentanone 1,5-bisphosphate (**10**). Infrared spectra of deuterium oxide solutions of the sodium salts of these ketose phosphates (Figure 1) clearly demonstrate the absence of carbonyl stretching frequencies, and hence the absence of significant proportions of the free keto forms,

(1) F. C. Hartman and R. Barker, *Biochemistry*, **4**, 1068 (1965).
(2) S. J. Reynolds, D. W. Yates, and C. I. Pogson, *Biochem. J.*, **122**, 285 (1971).
(3) M. M. de Maine and S. J. Benkovic, *Arch. Biochem. Biophys.*, **152**, 272 (1972).
(4) S. J. Benkovic, J. J. Kleinschuster, M. M. de Maine, and I. J. Siewers, *Biochemistry*, **10**, 4881 (1971).
(5) P. A. Benkovic, W. P. Bullard, M. de Maine, R. Fishbein, K. J. Schray, J. J. Steffens, and S. J. Benkovic, *J. Biol. Chem.*, **249**, 930 (1974).
(6) C. J. Marcus, *J. Biol. Chem.*, **251**, 2963 (1976).
(7) C. F. Midelfort, R. K. Gupta, and I. A. Rose, *Biochemistry*, **15**, 2178 (1976).
(8) G. R. Gray and R. Barker, *Biochemistry*, **9**, 2454 (1970).

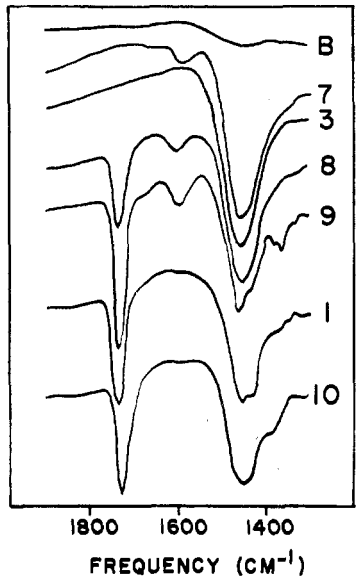
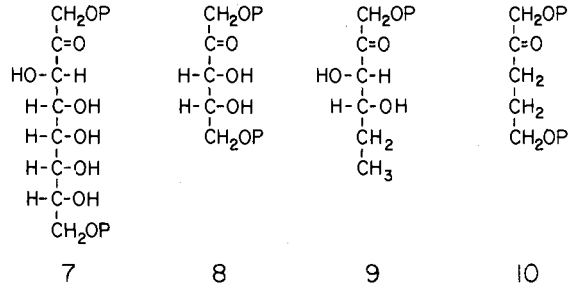


Figure 1. The infrared spectra in the 1300–1900-cm^{-1} region of deuterium oxide solutions of 1,3-dihydroxy-2-propanone phosphate (1), D-fructose 1,6-bisphosphate (3), D-*glycero*-D-*altro*-octulose 1,8-bisphosphate (7), D-*erythro*-pentulose 1,5-bisphosphate (8), 5,6-dideoxy-D-*threo*-hexulose 1-phosphate (9), and 1,5-dihydroxy-2-pentanone 1,5-bisphosphate (10) at pH 7, 25 °C.

in solutions of D-fructose 1,6-bisphosphate (3) and D-*glycero*-D-*altro*-octulose 1,8-bisphosphate (7), both which have the potential to exist in cyclic hemiacetal



forms. In contrast, the other ketose phosphates, which cannot exist in cyclic hemiacetal forms, have intense absorptions in the carbonyl stretching frequency region, demonstrating the presence of free carbonyl forms.

These results were confirmed by ^1H NMR spectroscopy. The inductive effect, as well as the diamagnetic anisotropy of the C-2 carbonyl group, leads to the deshielding of the C-1 and C-3 hydrogens. Consequently, the C-1 and C-3 hydrogen resonances occur at lower field in the carbonyl form than in the hydrated carbonyl or hemiacetal forms. In the spectrum of 1,3-dihydroxy-2-propanone phosphate (Figure 2) the hydrogen resonances of the free and hydrated carbonyl forms are readily observed. The doublet due to the C-1 hydrogens of the free carbonyl form (1) occurs 0.73 ppm to lower field than in the hydrated carbonyl form (2), and the C-3 hydrogen singlet of 1 similarly resonates 0.93 ppm to lower field than in **2**. The C-1 and C-3 resonances of the hydrated carbonyl form (2), as expected, have virtually the same chemical shifts as the corresponding resonances of the dimethyl acetal (Figure 2, inset). The other acyclic ketose phosphates examined (8–10) gave similar results: resonances ascribable to the C-1 and C-3 hydrogens of both the free and hydrated carbonyl forms were observed, and in each case the free carbonyl form was predominant. Resonances ascribable to the C-1 and

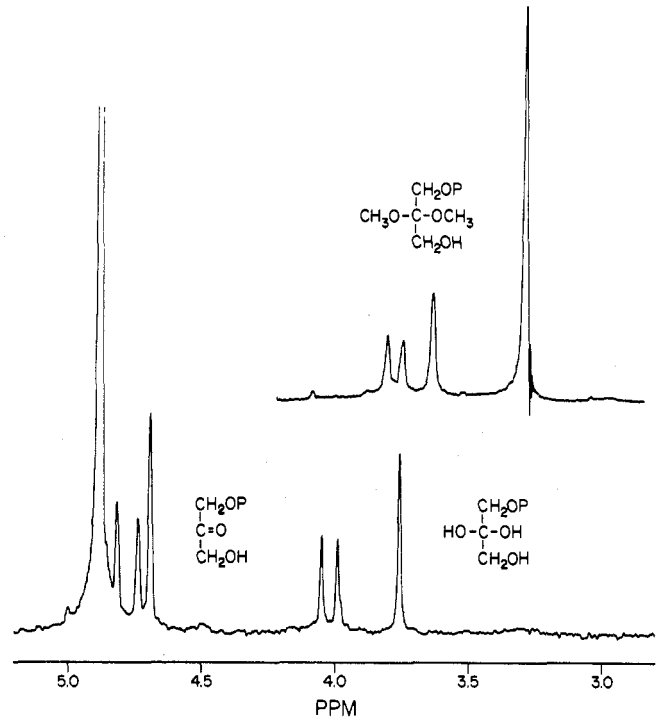


Figure 2. Proton magnetic resonance spectra (100 MHz) of the sodium salts of 1,3-dihydroxy-2-propanone phosphate (1, 2) in deuterium oxide, pH 4.8, and 1,3-dihydroxy-2-propanone phosphate dimethyl acetal in deuterium oxide, pH 7.2. Chemical shifts are expressed in ppm downfield from internal 3-(trimethylsilyl)propanesulfonic acid sodium salt.

Table I
Percentage of the Keto Form in Solutions of the Sodium Salts of Ketose Phosphates [a]

Ketose phosphate	% keto	
	Ir	NMR
1,3-Dihydroxy-2-propanone phosphate (1)	55 (25 °C)	63 (37 °C)
D-Fructose 1,6-bisphosphate (3)	1.7 (25 °C)	Not detected
D-*glycero*-D-*altro*-Octulose 1,8-bisphosphate (7)	0	Not detected
D-*erythro*-Pentulose 1,5-bisphosphate (8)	84 (25 °C)	Not determined
5,6-Dideoxy-D-*threo*-hexulose 1-phosphate (9)	96 (25 °C)	91 (28 °C)
1,5-Dihydroxy-2-pentanone 1,5-bisphosphate (10)	84 (25 °C)	84 (28 °C)

[a] Taken from ref 8.

C-3 hydrogens of the free carbonyl forms of D-fructose 1,6-bisphosphate (3) and D-*glycero*-D-*altro*-octulose 1,8-bisphosphate (7) were not observed, however, confirming infrared data.

The exact proportion of the free carbonyl form present in solutions of these ketose phosphates was obtained either by integration of the NMR spectrum or by measurement of the extinction coefficient of the carbonyl stretching frequency in solutions of known concentration. As seen in Table I, data obtained by the two methods are in very good agreement. The free carbonyl form is strongly preferred over the *gem*-diol form in solutions of the acyclic ketose phosphates examined, but acyclic forms are not present to a significant extent where the potential exists to form cyclic hemiacetals. Amplification of the ir spectrum of D-fructose 1,6-bis-

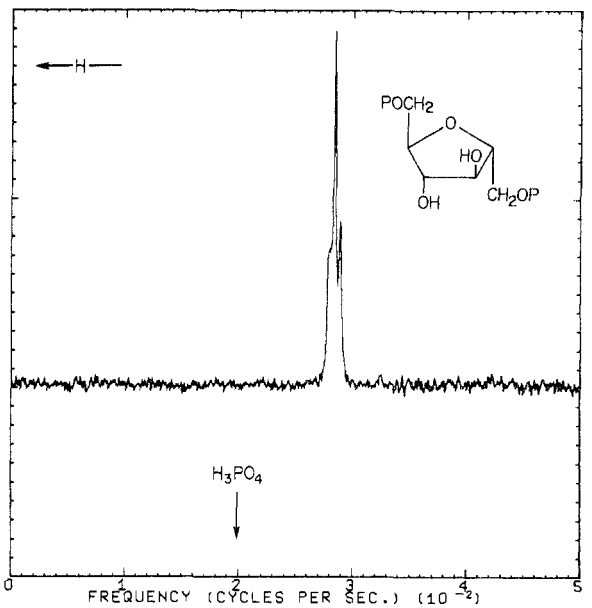


Figure 3. ^{31}P nuclear magnetic resonance spectrum of 2,5-anhydro-D-mannitol 1,6-bisphosphate (**14**), pH 7.0.

phosphate revealed that 1.7% of the keto form (**3**) was present, but the free carbonyl form could not be detected in solutions of D-*glycero*-D-*altro*-octulose 1,8-bisphosphate. Solutions of 1,3-dihydroxy-2-propanone phosphate, however, contain 55% of the keto form at 25 °C, a value later confirmed by an independent technique.[2]

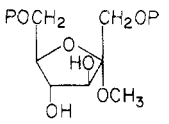
These conclusions strongly disagree, however, with those of other workers. Using the results of a study of D-fructose 1,6-bisphosphate by uv and ORD spectroscopy, McGilvery[9] concluded that the keto form (**3**) was predominant in solution, and, employing similar methods, Avigad et al.[10] concluded that approximately 20% of the keto form (**3**) was present. The latter workers, ignoring the possibility that *gem*-diol forms might be present, also concluded that 1,3-dihydroxy-2-propanone phosphate exists solely in the keto form (**1**) in solution. In order to resolve the differences between these studies, Swenson and Barker[11] examined deuterium oxide solutions of several aldoses and ketoses by infrared, ultraviolet, and circular dichroic spectroscopy and convincingly demonstrated that only infrared spectroscopy is a reliable index of the proportion of free carbonyl tautomer present in solution. Ultraviolet spectra showed principally impurities, and ORD spectra could not be interpreted quantitatively.

Clearly, either ir or ^1H NMR spectroscopy can be reliably used to determine the proportions of free carbonyl and *gem*-diol forms in solution. Proton magnetic resonance spectroscopy is most useful where resonances attributable to the two forms are easily identified and where more than trace quantities of either form are present. Infrared spectroscopy of deuterium oxide solutions, on the other hand, can readily detect small quantities of free carbonyl forms.

Characterization of Cyclic Forms

Cyclic forms of the aldoses have been characterized principally by ^1H NMR spectroscopy[12-14] because of the ease of identification of the anomeric C-1 hydrogen resonances of the α- and β-furanose and -pyranose forms. Because keto sugars lack an anomeric hydrogen, their cyclic forms are not as readily identified by ^1H NMR spectroscopy. Their characterization awaited the development of NMR techniques for observing other nuclei.

Through a comparison of the ^{31}P NMR spectra of D-fructose 1,6-bisphosphate, methyl α-D-fructofuranoside 1,6-bisphosphate (**11**), and methyl β-D-fructofuranoside 1,6-bisphosphate (**12**), Gray and Barker[8]



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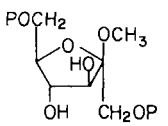

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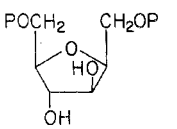
concluded that the β-furanose form (**6**) of D-fructose 1,6-bisphosphate was predominant over the α-form (**5**) in solution. The ^{13}C NMR spectrum of D-fructose 1,6-bisphosphate also indicated that one major form was present, but the resonances were not assigned.

In a more thorough examination of D-fructose 1,6-bisphosphate and related analogs by ^{31}P NMR spectroscopy, Gray[15] confirmed that the β-furanose form (**6**) was the major form in solution. Two types of analogs were examined: the methyl α- and β-glycosides of D-fructose 1,6-bisphosphate (**11** and **12**, respectively) and the configurationally related anhydroalditol bisphosphates **13** and **14**. 2,5-Anhydro-D-glucitol 1,6-bisphos-



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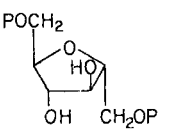

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phate (**13**) and 2,5-anhydro-D-mannitol 1,6-bisphosphate (**14**) differ from the α- and β-furanose forms of D-fructose 1,6-bisphosphate (**5** and **6**, respectively) only by the substitution of a hydrogen for the anomeric hydroxyl group at C-2. In the ^{31}P NMR spectrum of **14** (Figure 3) a single triplet is observed because the phosphorus atoms are stereochemically equivalent. The spectrum of **13** (Figure 4), however, is more complex. One of the phosphorus resonances has the same chemical shift, and apparently the same coupling constant to the methylene hydrogens (J = 5 Hz), as the phosphorus nuclei of **14**, but the other phosphorus resonance of **13** has a larger coupling constant (J = 6.8 Hz) and is shifted 0.5 ppm to lower field. The resonance at lower field in the spectrum of **13** can be assigned to the C-1 phosphate group, which differs from the phosphate group at C-6 only in that it is cis to the neighboring hydroxyl group.

A similar downfield shift was observed for the C-1 phosphorus resonance of **11** relative to **12**. The ^{31}P NMR spectrum of **12** (Figure 5) contains two overlapping triplets. The triplet at higher field is poorly re-

(9) R. W. McGilvery, *Biochemistry*, 4, 1924 (1965).
(10) G. Avigad, S. Englard, and I. Listowsky, *Carbohyd. Res.*, 14, 365 (1970).
(11) C. A. Swenson and R. Barker, *Biochemistry*, 10, 3151 (1971).
(12) R. U. Lemieux and J. D. Stevens, *Can. J. Chem.*, 44, 249 (1966).
(13) S. J. Angyal, *Angew. Chem., Int. Ed. Engl.*, 8, 157 (1969).
(14) B. Coxon, *Adv. Carbohyd. Chem. Biochem.*, 27, 7 (1972).
(15) G. R. Gray, *Biochemistry*, 10, 4705 (1971).

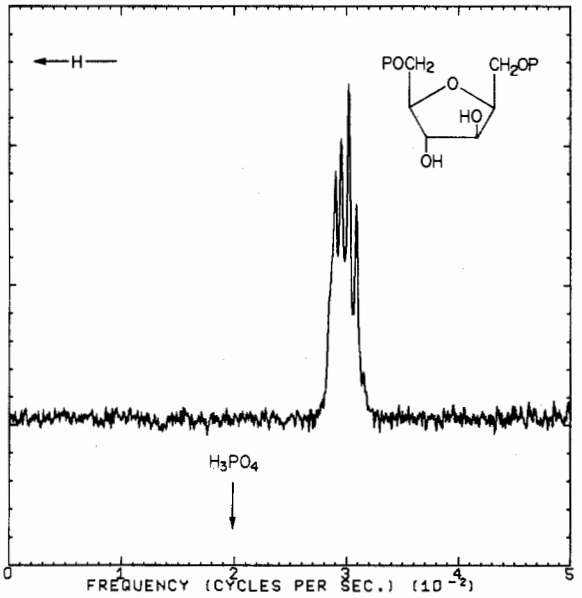


Figure 4. ^{31}P nuclear magnetic resonance spectrum of 2,5-anhydro-D-glucitol 1,6-bisphosphate (**13**), pH 7.6.

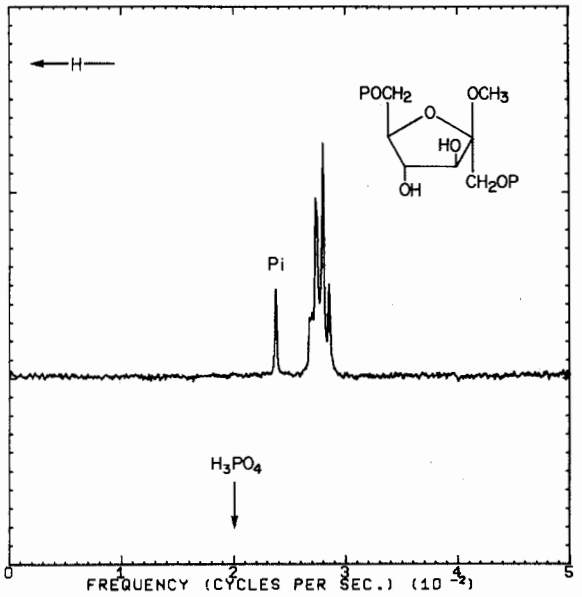


Figure 5. ^{31}P nuclear magnetic resonance spectrum of methyl β-D-fructofuranoside 1,6-bisphosphate (**12**), pH 7.0.

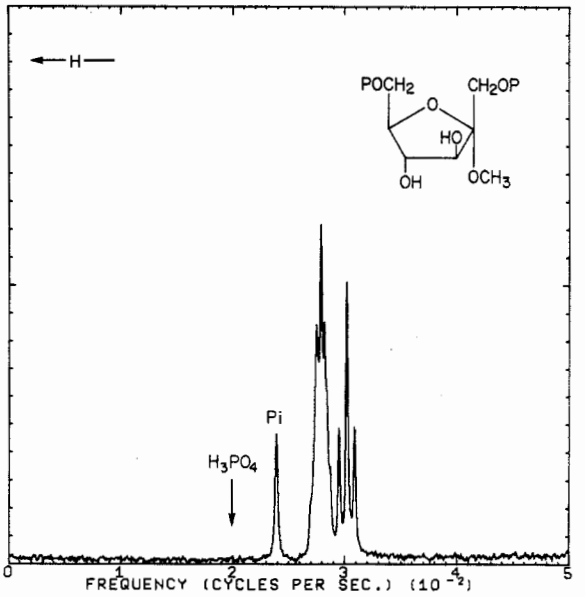


Figure 6. ^{31}P nuclear magnetic resonance spectrum of methyl α-D-fructofuranoside 1,6-bisphosphate (**11**), pH 7.0. The sample contains 25% β anomer (**12**).

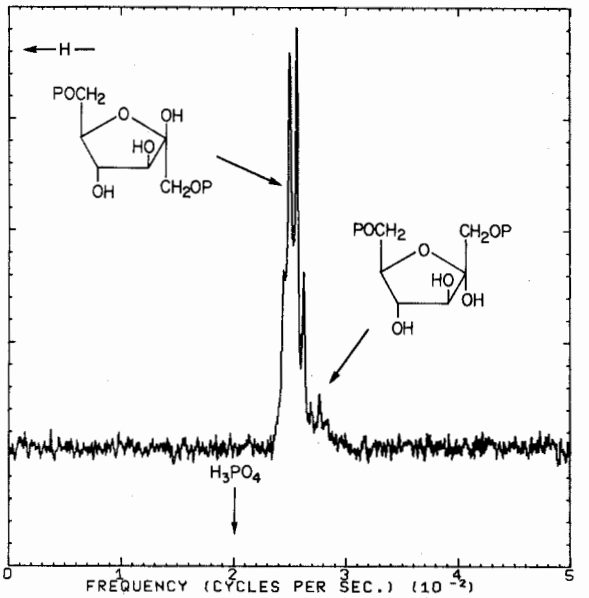


Figure 7. ^{31}P nuclear magnetic resonance spectrum of D-fructose 1,6-bisphosphate (**5, 6**), pH 6.15.

solved, has a small coupling constant (J = 4.9 Hz), and is assigned to the C-6 phosphorus resonance, and the triplet at lower field (J = 5.5 Hz) is assigned to the C-1 phosphorus resonance. In the ^{31}P NMR spectrum of **11** (Figure 6), the C-1 phosphorus resonance has a larger coupling constant (J = 7.1 Hz) and is shifted downfield relative to the C-6 phosphorus resonance, again indicative of the cis relationship with the C-3 hydroxyl group.

The ^{31}P NMR spectrum of D-fructose 1,6-bisphosphate (Figure 7) contains resonances ascribable to both the β-furanose (**6**) and α-furanose (**5**) forms. The weak triplet at lower field can be assigned to the 1-phosphate group of the α-furanose form (**5**) because of its chemical shift and J_{PH} value, and the two intense overlapping triplets can be assigned to the phosphate groups of the predominant β-furanose form (**6**).

The assignments of these resonances were confirmed by the synthesis of D-fructose-*6,6-d*$_2$ 1,6-bisphosphate.[15,16] In the ^{31}P NMR spectrum of the deuterated analog (Figure 8), the C-6 phosphorus resonances of both furanose forms occur as a broadened singlet at higher field than the triplets of the C-1 phosphorus resonances of the α- and β-furanose forms.

The furanose structures of D-fructose 1,6-bisphosphate were later identified by ^{13}C NMR spectroscopy.[17,18] A comparison of the ^{13}C resonances of D-fructose 1,6-bisphosphate and D-fructose 6-phosphate with those of known α- and β-fructofuranose residues[19]

(16) G. R. Gray and R. Barker, *Carbohyd. Res.*, **20**, 31 (1971).
(17) S. J. Benkovic, J. L. Engle, and A. S. Mildvan, *Biochem. Biophys. Res. Commun.*, **47**, 852 (1972).
(18) T. A. W. Koerner, Jr., L. W. Cary, N. S. Bhacca, and E. S. Younathan, *Biochem. Biophys. Res. Commun.*, **51**, 543 (1973).
(19) A. Allerhand and D. Doddrell, *J. Am. Chem. Soc.*, **93**, 2777, 2779 (1971).

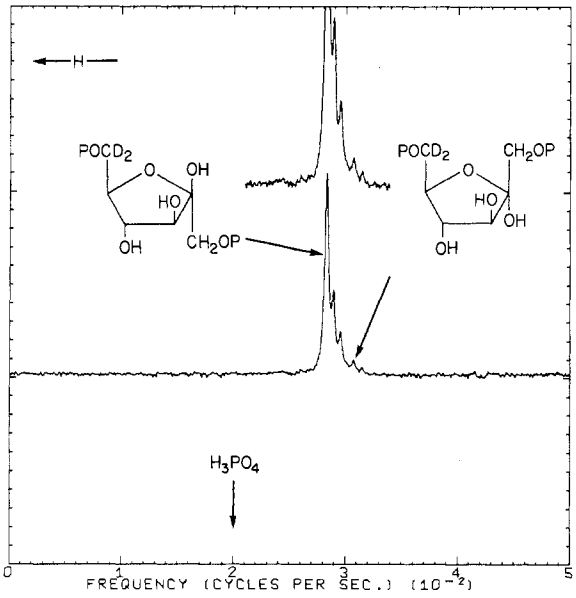


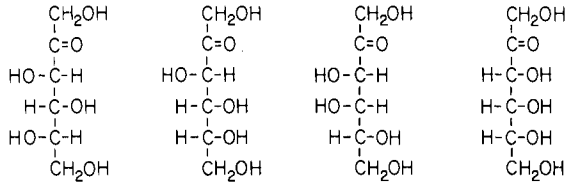
Figure 8. ^{31}P nuclear magnetic resonance spectrum of 6,6-d_2 D-fructose 1,6-bisphosphate, pH 7.0.

demonstrated that both phosphate esters exist predominantly (~80%) in the β-furanose form in solution. The free carbonyl forms were not observed.[18] The free carbonyl forms of D-fructose 1,6-bisphosphate and D-fructose 6-phosphate have been observed, however, in recent studies employing uniformly ^{13}C-enriched samples.[7] The free keto form of D-fructose 6-phosphate (4 ± 0.4%) was observed in aqueous solution at 17 °C, but the free keto form of D-fructose 1,6-bisphosphate was not observed under the same conditions, due to line broadening caused by rapid mutarotation. The free keto forms of both sugars were observed, however, in 20% Me_2SO. Under comparable conditions (−6 °C, pH 4.5, 20% Me_2SO), fructose 6-phosphate contained about 2.7% keto while fructose 1,6-bisphosphate contained 1.3% keto, a 2.1-fold difference. Assuming a 2.1-fold difference in aqueous solution, the authors concluded that solutions of fructose 1,6-bisphosphate contain 2.0% of the keto form.

The development of ^{13}C NMR spectroscopy has greatly aided in the structural characterization of carbohydrates and other complex molecules.[20] Studies of the aldopyranoses by Dorman and Roberts[21] and of the inositols by Dorman, Angyal, and Roberts[22] established that ^{13}C chemical shifts were heavily dependent on the proximity of substituents on the ring, and a set of quantitative correlations was developed for interpreting the ^{13}C NMR spectra of pyranose carbohydrates. 1,3-Diaxial interactions, in particular, were found to be important sources of chemical shift differences in these systems.

These studies were extended to furanose carbohydrates by Que and Gray,[23] who examined the configurationally related 1,4- and 2,5-anhydro polyols. Again, ^{13}C chemical shifts were found to be heavily dependent on the proximity of substituents about the ring. A cis relationship of vicinal hydroxyl groups was found to be the most important factor determining the ^{13}C chemical shifts of ring carbon atoms. Carbon atoms attached to vicinal hydroxyl groups were found to resonate 5–7 ppm to higher field when the groups were cis than when they were trans. A cis relationship of vicinal hydroxyl and hydroxymethyl groups also produced an upfield shift of the ^{13}C resonances, but the effect was not as pronounced as with vicinal cis hydroxyl groups.

The foregoing studies made possible a determination of the equilibrium composition of the ketohexoses. There are eight isomeric ketohexoses comprising four enantiomeric D,L pairs. Que and Gray[23] examined a member of each of these pairs, namely L-sorbose (**15**), D-fructose (**16**), D-tagatose (**17**), and D-psicose (**18**). The



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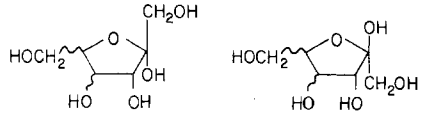
^{13}C NMR spectra of these analogs contained resonances readily ascribable to furanose and pyranose forms. The C-2 carbon atom was found to be the best probe of the tautomeric composition of these sugars, as the C-2 resonances of the various tautomers were well separated downfield from the other resonances. The C-2 resonances of furanose forms always occur downfield from the C-2 pyranose resonances,[24,25] and the chemical shifts of the C-2 furanose resonances readily identify the configuration at that center. When the C-2 and C-3 hydroxyl groups are cis, as in **19**, the C-2 resonance oc-



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curs 102–104 ppm downfield from Me_4Si, whereas when the C-2 and C-3 hydroxyl groups are trans, as in **20**, the C-2 resonance will occur 105–107 ppm downfield from Me_4Si. These shifts are observed regardless of the configuration elsewhere on the furanose ring.[23,25]

The C-2 resonances of the pyranose forms are assigned with greater difficulty. Que and Gray[23] based their assignments on a study of configurationally related 1,5-anhydroalditols, whereas other workers have utilized partially methylated or specifically deuterated derivatives.[21,22,25,26] The complexity of the task increases where the four possible tautomeric forms are observed in substantial proportions, as for example in the spectrum of psicose (Figure 9). At the present time, various groups of workers frequently disagree over the assignments of resonances, but are in agreement, however, with the assignments of the C-2 resonances and

(20) A. Allerhand, *Pure Appl. Chem.*, **41**, 247 (1975).
(21) D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.*, **92**, 1355 (1970).
(22) D. E. Dorman, S. J. Angyal, and J. D. Roberts, *J. Am. Chem. Soc.*, **92**, 1351 (1970).
(23) L. Que, Jr., and G. R. Gray, *Biochemistry*, **13**, 146 (1974).
(24) The C-2 resonances of the α- and β-furanose forms of tagatose were incorrectly assigned by Que and Gray.[23] As pointed out by Angyal and Bethell,[25] our tagatose sample was contaminated with L-sorbose. The peak at 94.8 ppm (upfield from CS_2) in the tagatose spectrum[23] is actually C-2 of α-L-sorbopyranose, not C-2 of β-D-tagatofuranose. The peak at 89.9 is C-2 of β-D-tagatofuranose.
(25) S. J. Angyal and G. S. Bethell, *Aust. J. Chem.*, **29**, in press.
(26) P. Herve du Penhoat and A. S. Perlin, *Carbohyd. Res.*, **36**, 111 (1974).

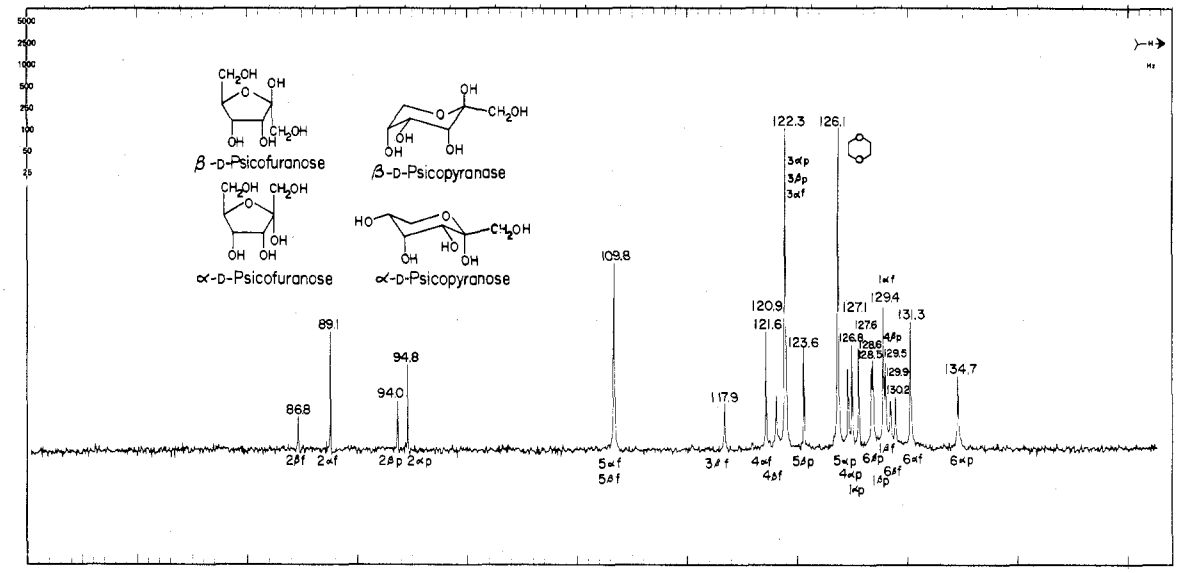

Figure 9. ^{13}C nuclear magnetic resonance spectrum of psicose at 25.1 MHz, 30 °C. Chemical shifts are expressed in ppm upfield from external CS_2 (internal dioxane at 126.1 ppm). Assignments other than C-2 are tentative.

hence with the proportions of the various tautomeric forms of the keto sugars in solution.

The Tautomeric Compositions of Keto Sugars and Their Phosphorylated Esters

The equilibrium compositions of D-fructose, D-fructose 1,6-bisphosphate, D-fructose 6-phosphate, D-psicose, L-sorbose, and D-tagatose are summarized in Table II. In this laboratory all values were obtained by integration of the anomeric ^{13}C resonances at tenfold horizontal scale expansion. With the exception of psicose, pyranose forms are more abundant, reflecting the greater stability of the six-membered ring. Psicose is particularly unusual since it is the only monosaccharide observed to date in which furanose forms are predominant in solution.

Interestingly, both D-fructose 1,6-bisphosphate and D-fructose 6-phosphate, which cannot exist in pyranose forms, exist in the same α:β furanose ratio (1:4.5) as fructose. Apparently, phosphorylation does not alter the relative stability of the α- and β-furanose forms. The α:β furanose ratio appears to be determined principally by the configuration of hydroxyl groups at C-2 and C-3. In every case (Table II) the anomeric C-2 hydroxyl group is cis to the C-3 hydroxyl group in the predominant furanose anomer. This observation is in contrast to the finding that the anomeric hydroxyl group and the C-2 hydroxyl group are always trans in the predominant furanose anomers of aldoses.[13] This discrepancy is readily explained, however, because it is known that a cis arrangement of hydroxyl and hydroxymethyl groups in a furanose ring is very unfavorable.[13] In the furanose forms observed in this study, the C-2 hydroxymethyl group (or its phosphorylated ester) and the C-3 hydroxyl group are trans in the predominant anomer, indicating that, in a furanose ring, a cis interaction of vicinal hydroxyl and hydroxymethyl groups is more unfavorable than a cis interaction of vicinal hydroxyl groups.

Conclusions

The development of the Fourier transform technique has clearly made ^{13}C NMR the method of choice for determining the tautomeric compositions of carbohydrates in solution. The sensitivity of the method allows detection of minor forms, and the large range of chemical shifts observed for the anomeric carbon atom of the various tautomers makes assignment and accurate quantitative estimation possible. The assignment of all resonances of each tautomer is still not possible, however, and further work is needed in order to develop a more quantitative set of parameters for predicting ^{13}C chemical shifts in these systems.

The structures of D-fructose 1,6-bisphosphate and 1,3-dihydroxy-2-propanone phosphate in solution were correctly established before the availability of the ^{13}C NMR technique, demonstrating that ir and ^{1}H and ^{31}P

Table II
Proportions of Pyranose and Furanose Forms of Ketoses at Equilibrium in Aqueous Solutions [a]

Ketose	Furanose, % α	Furanose, % β	Pyranose, % α	Pyranose, % β	Temp, °C	Ref[b]
D-Fructose	9	31 ± 3	3	57 ± 6	36	19
	5	23	tr	72	30	23
	10	30	<5	60	*c*	26
	4	21	tr	75	27	25
D-Fructose 1,6-bisphosphate[d]	~10	~90			31	15
	20 ± 4	80 ± 10			29	17
	23 ± 4	77 ± 4			35	18
	15	81			*c*	7
	18	80			30	*f*
D-Fructose 6-phosphate[e]	20 ± 4	80 ± 10			29	17
	19	81			35	18
	18	78			30	*f*
D-Psicose	38	15	26	21	30	23
	40	10	25	25	*c*	26
	39	15	22	24	27	25
L-Sorbose	5	0	95	0	30	23
	0	0	100	0	*c*	26
	2	0	98	0	27	25
D-Tagatose	tr	5	78	17	30	23, 24
	0	0	90	10	*c*	26
	1	4	79	16	27	25

[a] ±2% unless otherwise indicated. [b] Chronological order for each ketose. [c] Not reported. [d] 1.7% of the keto form also present.[8] [e] 4.1–5.4% of the keto form also present.[7,11] [f] Obtained in this laboratory by integration of the anomeric ^{13}C resonances under the same conditions reported in ref. 23.

NMR spectroscopy are still useful structural characterization techniques in these systems. In fact, ir spectroscopy of deuterium oxide solutions of these keto sugars is the best way to quantitate and unambiguously identify small amounts of free carbonyl forms.

This work provides the basis on which the tautomeric compositions of the other keto sugars and their derivatives can be established and furnishes a starting point for those interested in exploring the mechanisms of enzymes which utilize substrates which exist as a mixture of tautomers.

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