resent the first detailed demonstration of semiconservative replication in this latter class of plasmids. It should be recognized the results were obtainable because the methods used preserve the CCC form of the plasmid DNA.

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^{13}C Nuclear Magnetic Resonance Spectra and the Tautomeric Equilibria of Ketohexoses in Solution†

Lawrence Que, Jr.,‡ and Gary R. Gray*

ABSTRACT: The proportions of pyranose and furanose forms at equilibrium in aqueous solutions of L-sorbose, D-fructose, D-tagatose, and D-psicose have been determined by ^{13}C nuclear magnetic resonance spectroscopy. The assignments of the ^{13}C resonances were based on a study of configurationally related 1,5-anhydrohexitols and 1,4- and 2,5-anhydropolyols. With the exception of D-psicose, pyranose forms predominate, and the observed conformations and $\alpha:\beta$ ratios of these forms are in good agreement with values calculated from the interaction energies of nonbonded substituents. The $\alpha:\beta$ ratios of the furanose forms are determined principally by the geometry of hydroxymethyl and hydroxyl groups at C-2 and C-3. In every case, the C-2 hydroxymethyl group and the C-3 hydroxyl group are trans in the predominant furanose anomer.

Because of their biological importance, the composition and conformation of sugars in solution have been the subjects of intense investigation. Monosaccharides exist in several tautomeric forms in solution at equilibrium, and a knowledge of the relative abundance of these forms is necessary in order to understand their chemical and enzymatic reactivities. The tautomeric composition of aldoses has been determined principally by proton magnetic resonance spectroscopy (Lemieux and Stevens, 1966; Angyal, 1969). This method relies on the fact that the anomeric proton signals appear at lower field than the other proton signals and have chemical shifts and coupling constants characteristic of the configuration and conformation of the ring form. The tautomeric composition is determined by integration of the anomeric proton signals.

The equilibrium composition of ketohexoses has not been determined. There are eight isomeric ketohexoses comprising four enantiomeric D,L pairs, and a member of each of these pairs was examined in this investigation. These were L-sorbose (**1**), D-fructose (**2**), D-tagatose (**3**), and D-psicose (**4**).[1] The absence of an anomeric proton has made it impossible to observe and identify the tautomeric forms of ketoses by proton

† From the Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455. *Received July 30, 1973.* This work was supported by grants from the University of Minnesota Graduate School and the Research Corporation.

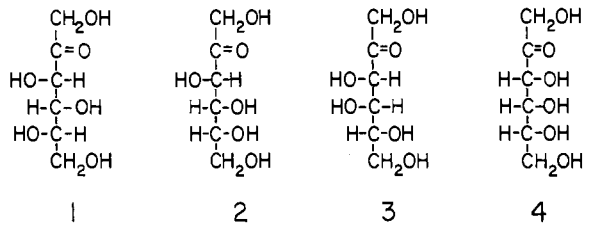
‡ Present address: Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

[1] The nomenclature of ketohexoses and other carbohydrates cited herein follows the IUPAC–IUB Rules for Carbohydrate Nomenclature [(1971), *Biochem. J. 125*, 673].

TABLE I: Chemical Shifts of the ^{13}C Resonances of 1,5-Anhydrohexitols.[a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6
1,5-Anhydro-D-glucitol (**5a**)	132.1	112.3	122.9	115.2	123.2	123.8
1,5-Anhydro-D-mannitol (**6a**)	131.5	112.1	125.4	119.1	123.6	122.8
1,5-Anhydro-D-altritol (**7a**)	131.3	116.4	127.8	123.2	123.2	126.5
1,5-Anhydro-D-talitol (**8a**)	130.0	113.2	124.4	123.0	123.5	122.0
1,5-Anhydro-L-galactitol (**9a**)[b]	131.3	113.2	123.5	118.5	126.2	123.5
1,5-Anhydro-L-allitol (**10a**)[c]	131.4	116.6	126.5	123.6	126.8	127.5

[a] In parts per million upfield from external CS_2. [b] Values obtained from the D isomer. [c] Calculated values; see discussion in text.



magnetic resonance (pmr) spectroscopy. Recently, however, ^{13}C nuclear magnetic resonance spectroscopy has been shown to be applicable to the study of the configuration and conformation of aldopyranoses (Dorman and Roberts, 1970a; Hall and Johnson, 1969) and the tautomeric compositions of fructose (Doddrell and Allerhand, 1971), and its phosphorylated forms (Koerner *et al.*, 1973). In order to use this technique to identify the tautomeric forms of ketohexoses, the ^{13}C resonances of each of these forms must be unequivocally assigned. Assignments of the ^{13}C resonances of the ketopyranose forms will be based on a study of the ^{13}C resonances of configurationally related 1,5-anhydrohexitols, utilizing the empirical constants derived by Dorman *et al.* for the ^{13}C resonances of inositols (1970). Assignments of the ^{13}C resonances of the ketofuranose forms will be based on a study of the ^{13}C resonances of configurationally related 1,4-anhydropentitols and 2,5-anhydrohexitols.

Experimental Procedure

Materials. D-Psicose was prepared by hydrolysis of 1,2:4,5-di-*O*-isopropylidene-β-D-psicopyranose as described by Tipson *et al.* (1969). Proton magnetic resonance spectroscopy indicated that complete hydrolysis had been achieved. 1,5-Anhydro-D-talitol was prepared by hydrolysis of methyl 2,6-anhydro-α-D-altroside as described by Rosenfeld *et al.* (1948), followed by reduction of the aldehyde with sodium cyanoborohydride at pH 4.0 (Borch *et al.*, 1971). L-Sorbose, D-fructose, and D-tagatose were obtained from commercial sources.

Natural Abundance ^{13}C Nuclear Magnetic Resonance (nmr) Spectra. Proton noise decoupled (Weigert *et al.*, 1968) ^{13}C nmr spectra were obtained at 25.1 MHz on a Varian XL-100-15-NMR spectrometer operating in the Fourier transform mode at 30°. 1,4-Dioxane was used as an internal reference. All chemical shifts are expressed in parts per million upfield from external carbon disulfide (1,4-dioxane at 126.1 ppm). Spectra of the ketohexoses were determined 24 hr after dissolution. Most samples were 1–2 M solutions in deuterium oxide. The resonances of methylene carbon atoms were assigned by off-resonance decoupling.

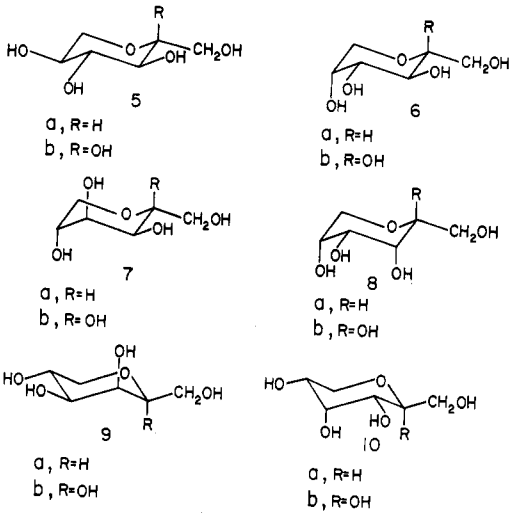
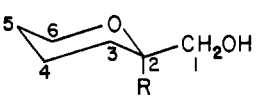


FIGURE 1: Pyranose forms of the ketohexoses (b) and their configurationally related 1,5-anhydrohexitols (a).

Results

^{13}C Nmr Spectra of 1,5-Anhydrohexitols. The ketopyranose forms which have been observed in this study, and their configurationally related 1,5-anhydrohexitol analogs, are shown in Figure 1. Structurally, these analogs differ from the corresponding ketopyranose forms only by the substitution of a hydrogen for the anomeric hydroxyl group at C-2. Based on the proximity correlations of Dorman *et al.* (1970), this substitution should allow an accurate prediction of the ^{13}C resonances of the corresponding ketopyranose forms to be made.

The ^{13}C chemical shifts of the 1,5-anhydrohexitols are summarized in Table I. For convenience in discussing these assignments, a uniform numbering system will be adopted, *i.e.* the carbon atoms of the 1,5-anhydrohexitols will be numbered to correspond with the respective carbon atoms of the ketopyranose forms (**11**). This numbering system follows the normal convention for ketose sugars, but is reversed for the 1,5-anhydrohexitols.[2]



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[2] Throughout this manuscript these derivatives will be referred to as 1,5-anhydrohexitols, but they are numbered as 2,6-anhydrohexitols so that a direct correspondence with their configurationally related pyranose forms is possible.

TABLE II: Chemical Shifts of the ^{13}C Resonances of 1,4- and 2,5-Anhydropolyols.[a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6
1,4-Anhydroerythritol (**12a**)	121.3	121.8	121.8	121.3		
1,4-Anhydroribitol (**12b**)	120.5	121.7	121.1	110.9	131.3	
1,4-Anhydroallitol (**12c**)	120.8	121.2	120.7	110.7	121.4	130.4
1,4-Anhydrolyxitol (**13a**)[b]	121.5	121.5	122.2	111.9	132.3	
1,4-Anhydromannitol (**13b**)	122.4	121.3	121.7	112.5	123.3	129.6
1,4-Anhydroxylitol (**14a**)	120.0	116.0	116.4	111.8	132.8	
1,4-Anhydroglucitol (**14b**)	119.3	116.3	116.8	112.8	123.7	129.1
1,4-Anhydrothreitol (**15a**)	119.8	116.4	116.4	119.8		
1,4-Anhydroarabinitol (**15b**)	119.5	115.7	114.6	107.1	131.1	
2,5-Anhydroiditol (**14c**)[c]	132.6	112.3	116.0	116.0	112.3	132.6
2,5-Anhydroglucitol (**15c**)[c]	132.5	111.7	115.7	114.6	107.9	131.0
2,5-Anhydromannitol (**15d**)[c]	131.7	110.4	116.3	116.3	110.4	131.7

[a] In parts per million upfield from external CS_2. [b] 2,5-Anhydro-D-arabinitol is the correct systematic name for **13a**. [c] C-3 and C-4 of these analogs correspond structurally with C-2 and C-3 of the other analogs reported in the table.

Inspection of Table I reveals that the ^{13}C resonances of 1,5-anhydrohexitols are very dependent on the proximity of substituents about the ring. These effects were first observed by Dorman *et al.* (1970) for the ^{13}C resonances of inositols and were shown to be reducible to a set of additive empirical constants which could be used to calculate the chemical shift of a carbon atom as a function of the configuration of substituents about β, γ, and δ carbon atoms. In this system, the chemical shift of the α carbon atom is correlated with epimerization of hydroxyl groups about adjacent carbon atoms from the equatorial to the axial configuration. If the hydroxyl group on the α carbon is equatorial, these constants, expressed in parts per million, are designated βe, γe, and δe, and if the α carbon bears an axial hydroxyl group, they are designated βa, γa, and δa.

Using the ^{13}C resonances of **5a** (see Table I) as reference points (all substituents are equatorial), analogous constants can be derived for the 1,5-anhydrohexitols: $\beta a = -0.4$ ppm, $\gamma a = -0.5 \pm 0.4$ ppm, $\beta e = +3.6 \pm 1.0$ ppm, $\gamma e = +2.8 \pm 0.3$ ppm (δa and δe cannot be determined). These values indicate that an axial hydroxyl group on a β or γ carbon will cause a large upfield shift of the α carbon if the latter bears an equatorial hydroxyl group, but will cause a slight downfield shift of the α carbon if it bears an axial hydroxyl group. The same observations were made by Dorman *et al.* (1970) for the inositols and by Dorman and Roberts (1970a) for the aldopyranoses.

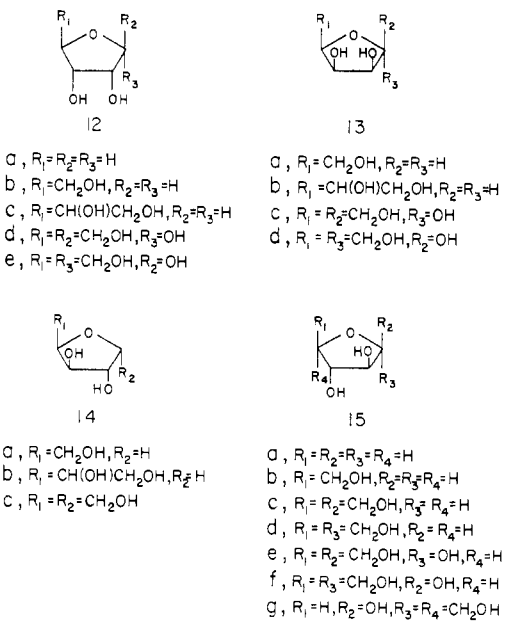

FIGURE 2: Furanose forms of the ketohexoses and their configurationally related 1,4- and 2,5-anhydropolyols.

In addition to these parameters, two other sets of empirical constants can be derived for the 1,5-anhydrohexitols. One set of these parameters, designated βo, γo, and δo, correlates the chemical shift of C-6 with epimerization of hydroxyl groups about β-, γ-, and δ-carbon atoms from the equatorial to the axial configuration, and the other set of parameters, designated βc, γc, and δc, correlates the chemical shift of C-2 (which bears an equatorial hydroxymethyl substituent) with the same changes in configuration about β-, γ-, and δ-carbon atoms. For example, the chemical shift of C-6 of **7a** differs from C-6 of **6a** by $\gamma o = +3.7$ ppm, and C-2 of **7a** differs from C-2 of **6a** by $\gamma c = +4.3$ ppm. Other values were obtained in a similar manner and were found to be: $\beta o = -1.3 \pm 0.3$ ppm, $\gamma o = +3.7$ ppm, $\delta o = -0.6 \pm 0.3$ ppm, $\beta c = +1.0 \pm 0.1$ ppm, $\gamma c = +4.3$ ppm, and $\delta c = 0.0$ ppm. The values of βc, γc, and δc are in excellent agreement with those obtained from the data of Dorman and Roberts (1970a) for the hexose aldopyranoses: $\beta c = +1.0 \pm 0.2$ ppm, $\gamma c = +4.2 \pm 0.5$ ppm, and $\delta c = 0.0$ ppm.

These data again emphasize the importance of steric effects on the chemical shifts of carbon atoms in six-membered rings and provide convincing arguments on which to base the assignments of the carbon resonances of the pyranose forms of the ketohexoses.

^{13}C Nmr Spectra of 1,4- and 2,5-Anhydropolyols. In order to establish the basis for ^{13}C chemical-shift differences in furanose ring systems, several types of analogs have been examined in which the anomeric hydroxyl group of the furanose form is replaced by a hydrogen atom. The configurations of these analogs, as well as their related ketofuranose forms, are summarized by generalized structures **12**, **13**, **14**, and **15** (Figure 2).[3] A comparison of the ^{13}C chemical shifts

[3] Most anhydroalditols used in this study were of the D configuration, but some were available only as DL mixtures. For convenience, structural formulas represent only the D forms.

TABLE III: Chemical Shifts of the ^{13}C Resonances of Ketohexopyranoses and Ketohexofuranoses.[a]

Compounds	C-1	C-2	C-3	C-4	C-5	C-6
α-L-Sorbopyranose (**5b**)	128.1 (−4.0)[b]	94.7 (−17.6)	118.7 (−4.2)	122.2 (+7.0)	123.2 (0.0)	130.9 (+7.1)
α-L-Sorbofuranose (**15g**)	128.6	90.7	117.3	116.6	114.9	132.0
β-D-Fructopyranose (**6b**)	129.4 (−2.1)	94.4 (−17.7)	123.0 (−2.4)	125.1 (+6.0)	123.5 (−0.1)	128.8 (+6.0)
α-D-Fructofuranose (**15e**)	129.7	88.0	110.6	116.5	111.3	131.6
β-D-Fructofuranose (**15f**)	129.9	90.9	118.1	117.1	111.9	130.3
α-D-Tagatopyranose (**9b**)	130.3 (−1.0)	94.3 (−18.9)	121.7 (−1.8)	122.8 (+4.3)	126.3 (−0.1)	128.7 (+5.2)
β-D-Tagatopyranose (**7b**)	129.1 (−2.2)	94.2 (−22.2)	123.4 (−4.4)	128.9 (+5.7)	123.2 (0.0)	132.4 (+5.9)
α-D-Tagatofuranose (**13c**)	*c*	89.9	115.9	*c*	113.3	131.5
β-D-Tagatofuranose (**13d**)	129.4	94.8	122.2	118.7	112.5	130.7
α-D-Psicopyranose (**10b**)	127.6 (−3.8)	94.8 (−21.8)	122.3 (−4.2)	127.1 (+3.5)	126.8 (0.0)	134.7 (+7.2)
β-D-Psicopyranose (**8b**)	128.6 (−1.4)	94.0 (−19.2)	122.3 (−2.1)	129.5 (+6.5)	123.6 (+0.1)	128.5 (+6.5)
α-D-Psicofuranose (**12d**)	129.4	89.1	122.3	120.9	109.8	131.3
β-D-Psicofuranose (**12e**)	129.9	86.8	117.9	121.6	109.8	130.2

[a] In parts per million upfield from external CS_2. [b] Values in parentheses represent the downfield or upfield shift in ppm from the corresponding resonance of the configurationally related 1,5-anhydrohexitol. [c] Obscured by other resonances.

of these analogs (Table II) reveals that the relative arrangement of vicinal hydroxyl groups on C-2 and C-3 of the 1,4-anhydro compounds and on C-3 and C-4 of the 2,5-anhydro compounds is the major factor contributing to chemical-shift differences of the ring carbon atoms. If the vicinal hydroxyl groups are cis, as in series **12** and **13**, the carbon atoms to which those hydroxyl groups are attached resonate 5–7 ppm to higher field than when they are trans (series **14** and **15**). The C-2 and C-3 resonances of series **12** and **13** analogs usually occur at 121–122 ppm, while the corresponding resonances of series **14** and **15** analogs occur at 115–117 ppm. A second factor which contributes to chemical-shift differences is the relative orientation of vicinal hydroxyl and hydroxymethyl groups. For example, the C-4 resonance of 1,4-anhydroribitol (**12b**) occurs 0.9 ppm to lower field than the C-4 resonance of 1,4-anhydroxylitol (**14a**), and the C-4 resonance of 1,4-anhydroarabinitol (**15b**) occurs 4.8 ppm to lower field than the C-4 resonance of 1,4-anhydrolyxitol (**13a**). A comparison of the C-4 chemical shifts of the other 1,4-anhydropolyols (and the C-2 and C-5 chemical shifts of the 2,5-anhydrohexitols) reveals the same trend, *i.e.* a cis arrangement of vicinal hydroxyl and hydroxymethyl groups will cause an upfield shift of the ^{13}C resonance of the carbon atom to which the hydroxymethyl group is attached. Interestingly, 1,3 interactions of hydroxyl and hydroxymethyl groups do not seem to be important causes of chemical-shift differences in these systems, as the C-2 resonances of 1,4-anhydroerythritol (**12a**), 1,4-anhydroribitol (**12b**), and 1,4-anhydrolyxitol (**13a**) are virtually identical.

^{13}C Nmr Spectra of Ketohexoses. The chemical shifts of the ^{13}C resonances of the pyranose and furanose forms of L-sorbose (**1**), D-fructose (**2**), D-tagatose (**3**), and D-psicose (**4**) are summarized in Table III. Given in parentheses for each resonance of a pyranose form is the downfield or upfield shift of that resonance relative to the corresponding resonance of the configurationally related 1,5-anhydrohexitol. For convenience in discussing these assignments, the spectrum of each ketohexose will be discussed separately.

D-Fructose (**2**). The ^{13}C nmr spectrum of D-fructose (Figure 3) contains 18 resonances divided into three sets of clearly defined intensity. The most intense set of resonances can be assigned to the β-pyranose form in the *1C* conformation (**6b**). A comparison of the resonances of **6b** with those of the configurationally related 1,5-anhydrohexitol (**6a**) reveals the expected chemical-shift changes due to introduction of the anomeric hydroxyl group at C-2. The C-1 and C-3 resonances of **6b** are shifted downfield slightly and the C-2 resonance is shifted downfield greatly, compared to the respective resonances of **6a**, due to the added inductive effect of the C-2 hydroxyl group (Roberts *et al.*, 1970), and the C-4 and C-6 resonances of **6b** are shifted upfield relative to the same resonances of **6a** because of 1,3-diaxial interaction with the axial C-2 hydroxyl group. As expected, the C-5 resonance of **6b** is unchanged relative to **6a** because of its location δ to C-2 (Dorman *et al.*, 1970, and see above).

Similar comparisons of the chemical shifts of the ^{13}C resonances of **5b**, **7b**, **8b**, and **9b** with the chemical shifts of the ^{13}C resonances of their configurationally related analogs (Table III) yield identical results. In all cases, the presence of the C-2 hydroxyl group in the pyranose form causes a 17–22-ppm downfield shift in the C-2 resonance, a 1–4-ppm downfield shift in the C-1 and C-3 resonances, a 4–7-ppm upfield shift in the C-4 and C-6 resonances, and no change in the C-5 resonance. Although the C-1 and C-6 assignments for some of the pyranose forms may be reversed, this will affect only the magnitude of those respective shifts and will clearly not change the overall assignment of the particular pyranose form.

The two remaining sets of resonances in the fructose spectrum can only be assigned to the α- and β-furanose forms (**15e** and **15f**). The resonances due to the ring carbons in these forms occur at much lower field than the resonances of pyranose ring carbons, in agreement with previous studies on the ribofuranosyl carbon atoms of nucleotides (Dorman and Roberts, 1970b; Kotowycz and Hayamizu, 1973) and the fructofuranosyl resonances of sucrose (Dorman and Roberts, 1971). The most intense set of these resonances is assigned to the β-furanose form because the C-2 and C-3 hydroxyl groups in this form are cis, causing an upfield shift of the C-2 and C-3 resonances of **15f** relative to the same resonances of **15e**. The other resonances of the β-furanose form (**15f**) are assigned by comparison with those of 2,5-anhydro-D-mannitol (**15d**) (Table II). The C-2 hydroxyl group of **15f** causes a slight downfield shift of C-1 (−1.8 ppm) and a large downfield shift of C-2 (−19.5 ppm), relative to the same resonances of **15d**, due to induction, but the effect of induction

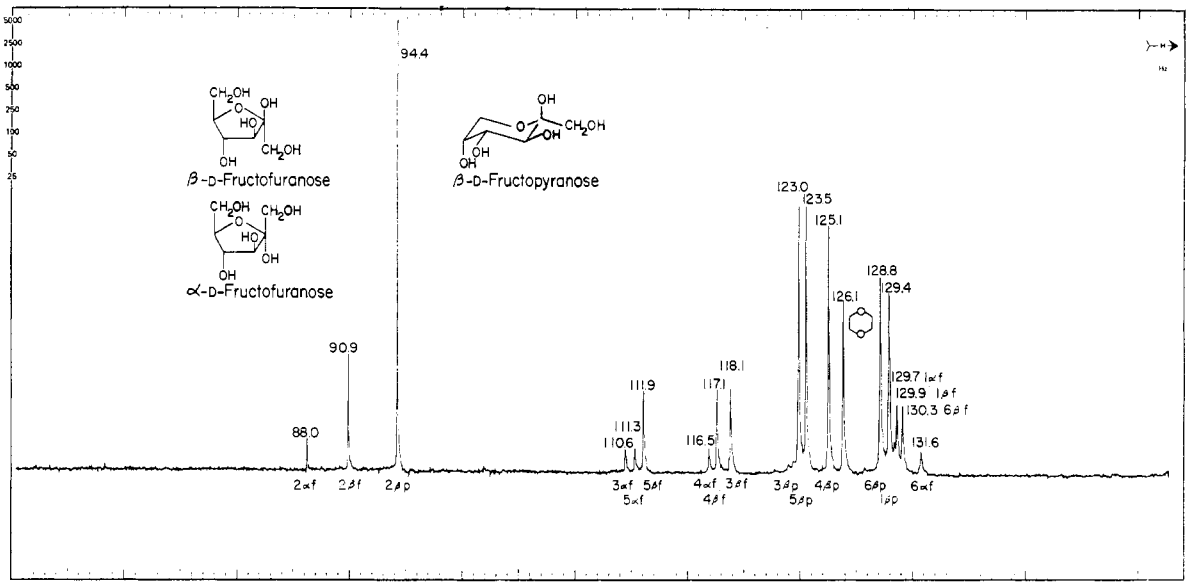

FIGURE 3: ^{13}C nuclear magnetic resonance spectrum of D-fructose (**2**): 2-sec acquisition time, 8-sec delay, 90-μsec pulse width, 8192 data points, 4580 transients. Major peaks occur at 88.0 (2αf), 90.9 (2βf), 94.4 (2βp), 110.6 (3αf), 111.3 (5αf), 111.9 (5βf), 116.5 (4αf), 117.1 (4βf), 118.1 (3βf), 123.0 (3βp), 123.5 (5βp), 125.1 (4βp), 128.8 (6βp), 129.4 (1βp), 129.7 (1αf), 129.9 (1βf), 130.3 (6βf), and 131.6 (6αf).

on the C-3 resonance of **15f** is countered by a larger upfield shift due to a cis interaction of vicinal hydroxyl groups. The C-4, C-5, and C-6 resonances of **15f** are only slightly affected by the C-2 hydroxyl group, and are approximately the same as those of **15d**. It might also be pointed out that the β-fructofuranose (**15f**) resonances have virtually the same chemical shifts as the β-fructofuranosyl resonances of sucrose (Dorman and Roberts, 1971).

The resonances of the α-furanose form (**15e**) are assigned by comparison with the corresponding resonances of 2,5-anhydro-D-glucitol (**15c**). The inductive effect of the C-2 hydroxyl group of **15e** causes a slight downfield shift of C-1 (−2.8 ppm) relative to C-1 of **15c**, but a greater downfield shift in the C-2 resonance (−23.7 ppm). In the latter case, the downfield shift caused by induction is not countered by an upfield shift due to the cis 1,2 interaction of hydroxyl groups observed in **15f**, so the observed downfield shift is greater. The C-3 resonance of **15e** is also shifted downfield relative to the same resonance of **15c**, and again this inductive effect is not countered by an upfield shift due to the cis 1,2 interaction of hydroxyl groups observed in **15f**. The C-4 and C-5 resonances of **15e** show some variance with those of **15c**, probably due to more subtle changes in preferred conformation. This seems reasonable since the C-5 resonance of **15c** is particularly low, as is the C-4 resonance of the configurationally related 1,4-anhydropentitol (**15b**).

L-Sorbose (**1**). The ^{13}C nmr spectrum of L-sorbose (Figure 4) contains two sets of six resonances each. The major set of resonances is assigned to the α-L-pyranose form in the *1C* (L) conformation, the known crystalline form of L-sorbose

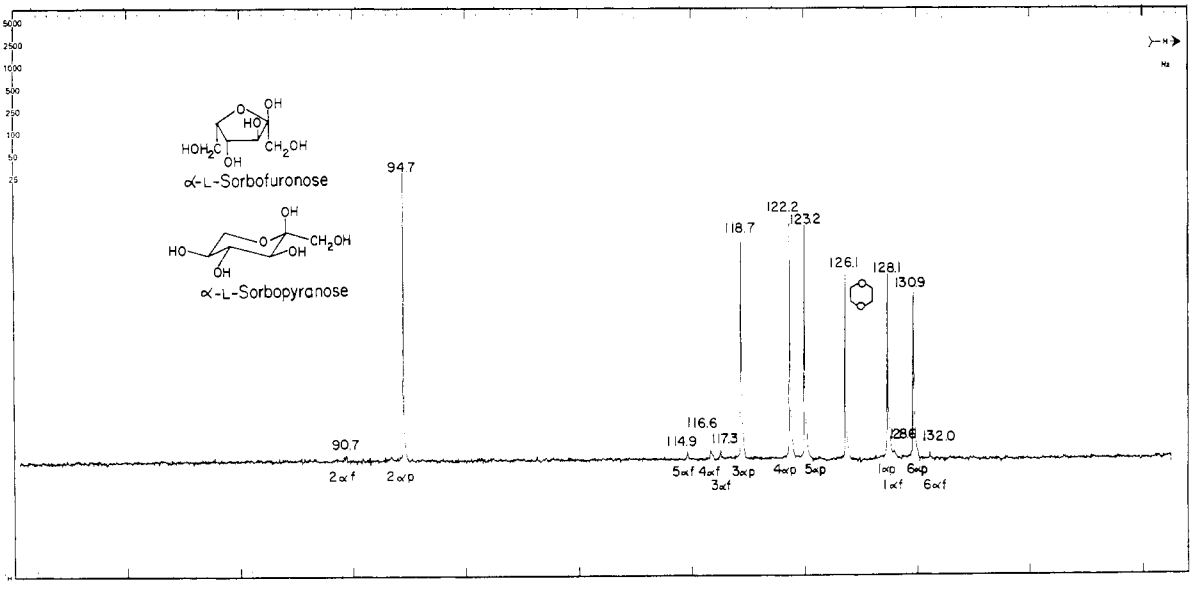

FIGURE 4: ^{13}C nuclear magnetic resonance spectrum of L-sorbose (**1**): 2-sec acquisition time, 2-sec delay, 30-μsec pulse width, 8192 data points, 6079 transients. Major peaks occur at 90.7 (2αf), 94.7 (2αp), 114.9 (5αf), 116.6 (4αf), 117.3 (3αf), 118.7 (3αp), 122.2 (4αp), 123.2 (5αp), 128.1 (1αp), 128.6 (1αf), 130.9 (6αp), and 132.0 (6αf).

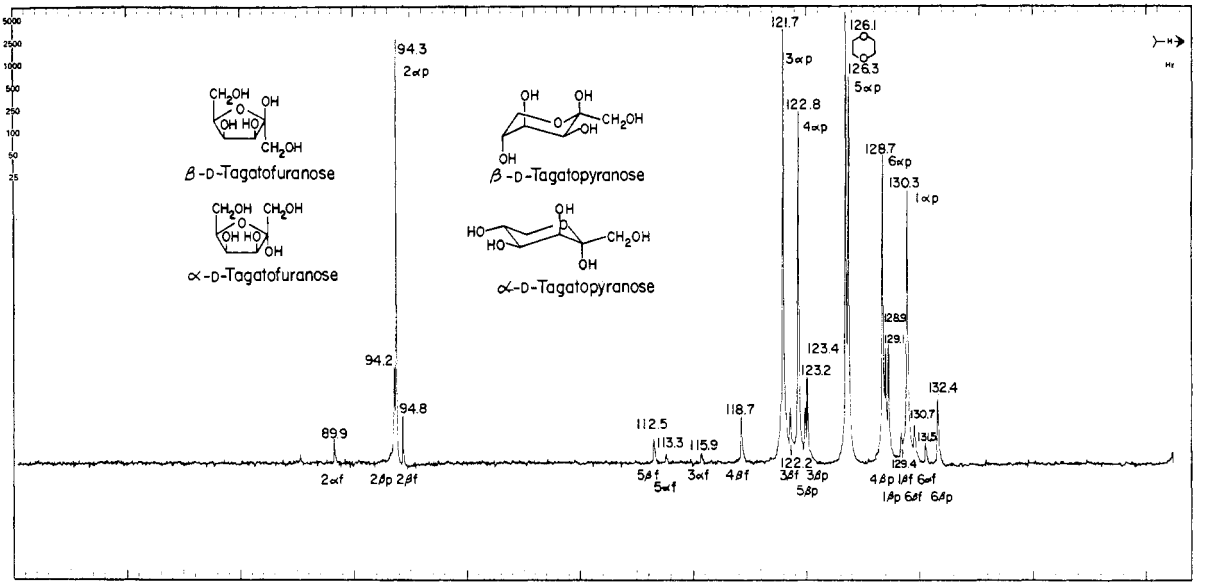

FIGURE 5: ^{13}C nuclear magnetic resonance spectrum of D-tagatose (**3**). 2-sec acquisition time, 2-sec delay, 30-μsec pulse width, 8192 data points, 17,528 transients. Major peaks occur at 89.9 (2αf), 94.2 (2βp), 94.3 (2αp), 94.8 (2βf), 112.5 (5βf), 113.3 (5αf), 115.9 (3αf), 118.7 (4βf), 121.7 (3αp), 122.2 (3βf), 122.8 (4αp), 123.2 (5βp), 123.4 (3βp), 126.3 (5αp), 128.7 (6αp), 128.9 (4βp), 129.1 (1βp), 129.4 (1βf), 130.3 (1αp), 130.7 (6βf), 131.5 (6αf), and 132.4 (6βp).

(Kim and Rosenstein, 1967). The resonances of this form are assigned through a comparison with the corresponding resonances of **5a**, the configurationally related 1,5-anhydrohexitol (Table III, see discussion under D-fructose).

The minor set of ^{13}C resonances in the sorbose spectrum is assigned to the α-L-furanose form (**15g**). The resonance at 90.7 ppm and those at 114–118 ppm are at too low a field to be assigned to a pyranose form. The chemical shift of the C-2 resonance (90.7 ppm) indicates that the hydroxyl groups at C-2 and C-3 are cis. In fact, the C-2 resonance of **15g** has the same chemical shift as the C-2 resonance of β-D-fructofuranose (**15f**) which differs structurally only in the configuration of the hydroxymethyl group at C-5. Comparing the ^{13}C chemical shifts of **15f** and **15g**, the only major differences are the C-5 resonances. As expected from the study of 1,4-anhydropolyol ^{13}C chemical shifts (see above) the cis relationship of the C-4 hydroxyl group and the C-5 hydroxymethyl group in **15g** shifts C-5 of **15g** upfield relative to C-5 of **15f**.

D-*Tagatose* (**3**). The ^{13}C nmr spectrum of D-tagatose (Figure 5) contains resonances ascribable to α- and β-pyranose and α- and β-furanose forms. The most intense set of resonances is assigned to the α-pyranose form in the *C1* conformation (**9b**) by a comparison with the resonances of 1,5-anhydro-L-galactitol (**9a**) and another set of resonances is easily assigned to the β-pyranose form in the *1C* conformation (**7b**) by a similar comparison with the resonances of 1,5-anhydro-D-altritol (**7a**) (see discussion under D-fructose). The C-6 chemical shift of **7b** is important in the latter comparison. β-D-

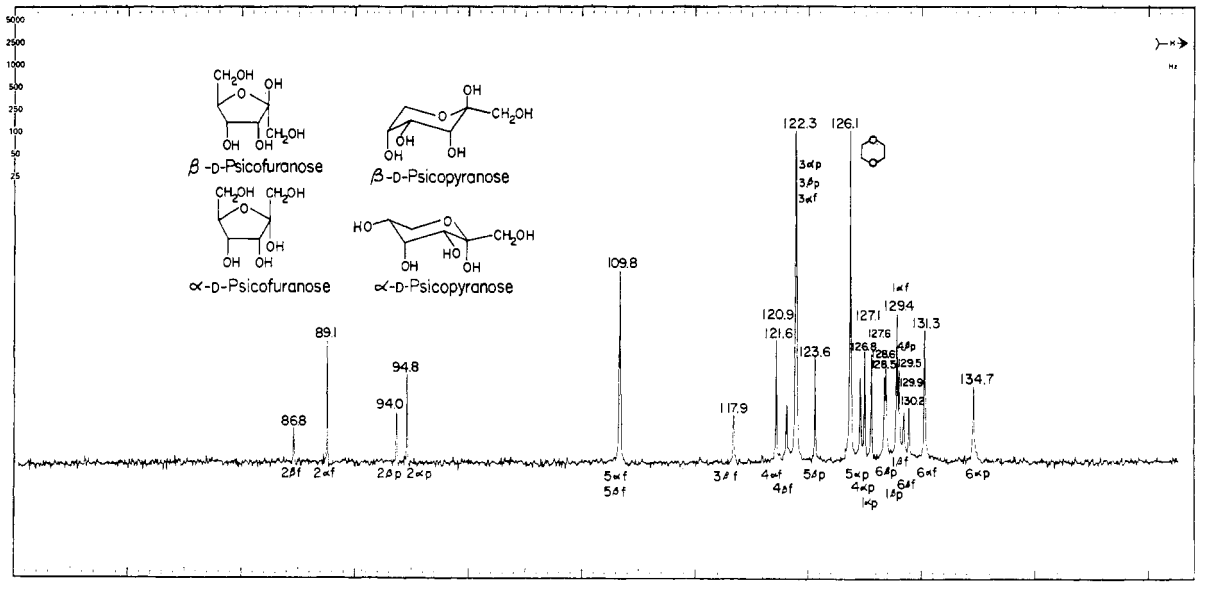

FIGURE 6: ^{13}C nuclear magnetic resonance spectrum of D-psicose (**4**): 1-sec acquisition time, 1-sec delay, 30-μsec pulse width, 4096 data points, 137,328 transients. Major peaks occur at 86.8 (2βf), 89.1 (2αf), 94.0 (2βp), 94.8 (2αp), 109.8 (5αf, 5βf), 117.9 (3βf), 120.9 (4αf), 121.6 (4βf), 122.3 (3αp, 3βp, 3αf), 123.6 (5βp), 126.8 (5αp), 127.1 (4αp), 127.6 (1αp), 128.5 (6βp), 128.6 (1βp), 129.4 (1αf), 129.5 (4βp), 129.9 (1βf), 130.2 (6βf), 131.3 (6αf), and 134.7 (6αp).

Tagatopyranose (**7b**) contains three axial hydroxyl groups, and two of these have 1,3-diaxial interactions with the axial C-6 hydrogen. As observed by Dorman *et al.* (1970) for the inositols, these effects are additive, and shift C-6 to an unusually high field (132.4 ppm).

The furanose forms of tagatose are assigned on the basis of the relative chemical shifts of the C-2 resonances. The resonance at lowest field (89.9 ppm) is assigned to C-2 of α-D-tagatofuranose (**13c**) in which the C-2 and C-3 hydroxyl groups are trans. The C-2 resonance of β-D-tagatofuranose (**13d**) is difficult to assign because of the close proximity of the two remaining resonances to the intense C-2 resonance of α-D-tagatopyranose (**9b**), but is probably the resonance at 94.8 ppm.

D-*Psicose* (**4**). The ^{13}C nmr spectrum of D-psicose (Figure 6) indicates that four forms are present in substantial proportions. The most intense set of resonances is assigned to the α-furanose form (**12d**). The C-2 resonance of **12d** (89.1 ppm) is shifted upfield relative to the C-2 resonance of **12e** (86.8 ppm) by the cis interaction between the C-2 and C-3 hydroxyl groups. The C-4 resonance of **12d** has the expected chemical shift (120.9 ppm) but the C-3 resonance is shifted upfield (122.3 ppm) by cis 1,2 interactions with both the C-2 and C-4 hydroxyl groups. The C-5 resonance of **12d** occurs at 109.8 ppm, reflecting the trans geometry of the C-4 hydroxyl and the C-5 hydroxymethyl groups, and the C-1 and C-6 resonances of **12d** occur at 129.4 and 131.3 ppm, respectively.

The resonances of β-D-psicofuranose (**12e**) are easily assigned and, as expected, differ substantially from those of **12d** only by the lower chemical shifts of C-2 and C-3. Because of the trans arrangement of the C-2 and C-3 hydroxyl groups in **12e**, the C-2 and C-3 resonances are shifted downfield to 86.8 and 117.9 ppm, respectively.

The resonances of β-D-psicopyranose (**8b**) are assigned by a comparison with those observed for 1,5-anhydrotalitol (**8a**) (see discussion under D-fructose), and the resonances of α-D-psicopyranose (**10b**) are assigned by a comparison with those calculated for 1,5-anhydroallitol (**10a**). The chemical shifts of the resonances of **10a** are calculated as follows: C-6 (**10a**) = C-6 (**5a**) + γo = 123.8 + 3.7 = 127.5 ppm; C-5 (**10a**) = C-5 (**5a**) + βe = 123.2 + 3.6 = 126.8 ppm; C-4 (**10a**) = C-4 (**7a**) − βa = 123.2 − (−0.4) = 123.6 ppm; C-3 (**10a**) = C-3 (**5a**) + βe = 122.9 + 3.6 = 126.5 ppm; C-2 (**10a**) = C-2 (**5a**) + γc = 112.3 + 4.3 = 116.6 ppm. The most notable feature of these calculations is the predicted chemical shift of the C-6 resonance. The C-6 resonance of **10a** is shifted upfield by a 1,3-diaxial interaction with the axial C-4 hydroxyl group. This upfield shift is also observed for the C-6 resonance of **7a**, and, in fact, the chemical shift of C-6 (**10a**) can be calculated from C-6 (**7a**): C-6 (**10a**) = C-6 (**7a**) − βo = 126.5 − (−1.3) = 127.8 ppm. The C-6 resonance of α-D-psicopyranose (**10b**) should therefore occur at an unusually high field because of 1,3-diaxial interactions with both the C-2 and C-4 hydroxyl groups as was observed for the C-6 resonance of β-D-tagatopyranose (**7b**). On this basis the 134.7-ppm resonance in Figure 6 can be assigned unequivocally to C-6 of α-D-psicopyranose (**10b**), the predominant pyranose form in solution.

Discussion

The equilibrium compositions of L-sorbose (**1**), D-fructose (**2**), D-tagatose (**3**), and D-psicose (**4**) in solution are tabulated in Table IV. With the exception of D-psicose, the pyranose forms are more abundant, reflecting the greater stability of the six-membered ring (Angyal, 1969). D-Psicose is particularly

TABLE IV: Proportions of Pyranose and Furanose Forms of Ketoses at Equilibrium in Aqueous Solutions at 30°.[a]

	Pyranose (%)		Furanose (%)	
Ketose	α	β	α	β
D-Fructose	0[b]	72	5	23
D-Psicose	26	21	38	15
L-Sorbose	95	0	5	0
D-Tagatose	71	15	5	9

[a] ±2%; based on peak areas of anomeric carbon atoms at tenfold horizontal scale expansion. [b] Peaks ascribable to this form were observed in the ^{13}C nmr spectrum, but probably represent 2% or less of the tautomeric composition.

TABLE V: Conformational Free Energies and the Calculated and Observed Conformations and Mole Fractions of Ketohexopyranoses at Equilibrium in Aqueous Solution.

	Conformation			Free Energy (kcal/mol)	Mole Fraction	
	Calcd[a]					
Ketose	*Cl* (%)	*lC* (%)	Obsd	Calcd[a]	Calcd[a]	Obsd
α-L-Sorbopyranose	0	100	*lC*	2.34	0.93	1.00
β-L-Sorbopyranose	38	62		3.83	0.07	
α-D-Fructopyranose	79	21		3.64	0.19	*b*
β-D-Fructopyranose	0	100	*lC*	2.80	0.81	1.00
α-D-Tagatopyranose	100	0	*Cl*	2.52	0.89	0.83
β-D-Tagatopyranose	30	70	*lC*	3.75	0.11	0.17
α-D-Psicopyranose	94	6	*Cl*	3.81	0.39	0.55
β-D-Psicopyranose	1	99	*lC*	3.56	0.61	0.45

[a] Calculated from interaction energies (Angyal, 1968). [b] Peaks ascribable to this form were observed in the ^{13}C nmr spectrum, but probably represent 2% or less of the tautomeric composition.

unusual since it is the only monosaccharide yet observed in which furanose forms are predominant in solution.

The proportion of each anomer present in solution at equilibrium depends on its relative free energy, but as yet no data are available which allow a prediction of these values to be made. The free energies of the pyranose forms, however, can be calculated. Each pyranose anomer can exist in two chair conformations, and for each of these conformations three rotational isomers exist because of rotation about the C-1–C-2 bond. The free energy of each of these forms can be calculated using the interaction energies derived by Angyal (1968). The free energy of each anomer is the weighted average of the free energies of the conformational isomers corrected for the entropy change on mixing (Eliel *et al.*, 1965). These values, as well as the calculated and observed conformation and mole fraction of each pyranose anomer, are tabulated in Table V. Clearly, these calculations correctly predict the conformation of each pyranose form observed in this study, and in addition provide reasonably good estimates of the conformational free energies, and hence the α:β pyranose ratios, of these pyranose forms. The calculations are in poorer agreement for fructose and psicose in which the α- and β-pyranose forms, respectively, are less stable than predicted.

The α:β furanose ratio is determined principally by the configuration of hydroxymethyl and hydroxyl groups at C-2 and C-3. In every case (Table IV) the C-2 and C-3 hydroxyl groups are cis in the predominant furanose anomer. This observation is in contrast to the finding that the C-1 and C-2 hydroxyl groups are always trans in the predominant furanose anomers of aldoses (Angyal, 1969). 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 (Angyal, 1969). In the furanose forms observed in this study, the C-2 hydroxymethyl group and the C-3 hydroxyl group are trans in the predominant isomer, 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.

These studies have established the tautomeric equilibria of the ketohexoses and have helped elucidate the structural factors which contribute to the relative stabilities of their pyranose and furanose forms. These data should allow a more precise interpretation of the chemical and enzymatic reactivities of these important carbohydrates to be made. In addition, these studies further demonstrate the applicability of ^{13}C nuclear magnetic resonance spectroscopy to the study of problems of conformational analysis, and furnish a basis from which the tautomeric equilibria and conformation of other sugars and their derivatives can be established.

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