The Structure of Biologically Important Carbohydrates. A ^{13}C Nuclear Magnetic Resonance Study of Tautomeric Equilibria in Several Hexulosonic Acids and Related Compounds

Thomas C. Crawford,* Glenn C. Andrews,* Herman Faubl, and Gwendolyn N. Chmurny

Contribution from Chemical Process Research, Central Research, Pfizer Inc., Groton, Connecticut 06340. *Received June 4, 1979*

Abstract: L-*xylo*-2-Hexulosonic acid (**3a**) (and its methyl ester, **3b**) in solution was shown to exist prodominantly in the α-pyranose form. D-*arabino*-2-Hexulosonic acid (and its sodium salt and methyl ester) was found to be a mixture of the α- and β-pyranose tautomers (**6, 7**) and the α- and β-furanose tautomers (**8, 9**). These results correlate very well with the known tautomers of L-sorbose (**3c**) and D-fructose (**6d, 7d, 8d, 9d**), respectively. The chemical shifts of the carbon resonances are only slightly affected by solvent changes. The tautomeric equilibrium of methyl D-*arabino*-2-hexulosonate was quite different in different solvents with the pyranose tautomer being favored in water and the α- and β-furanose tautomers being favored in Me_2SO. The rate of tautomerization of D-fructose in Me_2SO-d_6 was found to be much more rapid than the rate of tautomerization of methyl D-*arabino*-2-hexulosonate. D-*xylo*-5-Hexulosonic acid was found to exist as a mixture of the α- and β-furanose tautomers (**13a, 14a**) and the open-chain keto tautomer (**12a**) while D-*lyxo*-5-hexulosonic acid was found to exist as a mixture of the α- and β-furanose tautomers (**13c, 14c**) with little if any of the open-chain keto tautomer present. Sodium D-*threo*-2,5-hexodiulosonate and related compounds were shown to exist predominantly in a pyranose ring tautomer with the C-5 carbonyl hydrated. In all cases in which a crystal structure has been determined, the major tautomer in aqueous solution was found to be the same as in the crystal.

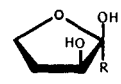
Discussion

During the past few years the use of ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR) has become important in the study of the conformation and tautomeric equilibria of sugars in solution.[1] Of particular importance has been the study of the tautomeric equilibrium of ketoses by ^{13}C NMR since alternative methods do not provide the desired information. The first ketoses studied by ^{13}C NMR were D-fructose[1,2] and D-turanose.[2] The spectrum of D-fructose revealed[2] the presence of four components whose gross structures were assigned and equilibrium was determined. Koerner and coworkers[3] and others[4] have reported the tautomeric composition of D-fructose 6-phosphate and D-fructose 1,6-diphosphate, and corrected several misassignments made by Doddrell and Allerhand[2] in the first detailed analysis of D-fructose by ^{13}C NMR. Subsequently Perlin et al.[5-7] studied a variety of furanose and hexose sugars as well as model compounds and showed that for the two possible furanose forms **1** and **2** the shift of the anomeric carbon of **1** was shielded by 4-7 ppm relative to the anomeric carbon of **2**.

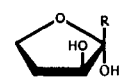
Que and Gray[8] have studied a number of ketohexoses in solution and also detailed this cis effect. Angyal and coworkers[9,10] have reported the most detailed study of hexuloses in solution as well as a study of 1-deoxyhexuloses and 3-hexuloses. In addition Funcke and Klemer[11] have reported the equilibrium mixture present in D-fructose and 1-amino-1-deoxy-D-fructose derivatives. Finally Hyvönen and co-workers[12] looked at the effect of temperature and concentration on the tautomeric equilibrium of D-fructose in water.

At the present time no data on the tautomeric equilibrium of hexulosonic acids has been reported. Owing to the commercial importance of D-*arabino*-2-hexulosonic acid [an intermediate in the preparation of D-*erythro*-hex-2-enono-1,4-lactone (or D-erythorbic acid)][13,14] and L-*xylo*-2-hexulosonic acid [an intermediate in the preparation of L-*threo*-hex-2-enono-1,4-lactone (or L-ascorbic acid)],[15] the potential value of D-*xylo*-5-hexulosonic acid[16] and D-*threo*-2,5-hexodiulosonic acid[17-21] in the synthesis of L-ascorbic acid, and the potential role a number of these compounds may play in the biosynthesis of L-ascorbic acid,[22] we initiated a program to determine the conformation and tautomeric equilibria of the above acids, the corresponding salts and esters, and a number of related compounds via ^{13}C NMR. The results of this study are reported here and are compared with the results that some of the above workers obtained on hexuloses and modified hexuloses.

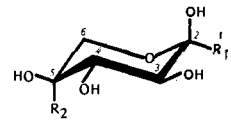
L-*xylo*-2-Hexulosonic Acid. The first molecule studied was L-*xylo*-hexulosonic acid and its methyl ester. In all solvents used (Me_2SO-d_6, D_2O, pyridine-d_5, DMF-d_7) only two tautomers were detected and one of those was present in very small amounts. The major tautomeric form was the α-pyranose tautomer shown by structures **3a** and **3b.** Carbons in **3a** and **3b** were assigned by use of the results of single-frequency off-



1

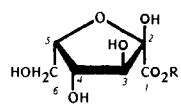


2



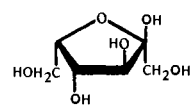
3

a. $R_1 = CO_2H, R_2 = H$
b. $R_1 = CO_2CH_3, R_2 = H$
c. $R_1 = CH_2OH, R_2 = H$
d. $R_1 = CH_3, R_2 = H$
e. $R_1 = CO_2CH_3, R_2 = D$



4

a. R = H
b. $R = CH_3$



5

0002-7863/80/1502-2220$01.00/0

Table I. ^{13}C Chemical Shifts of L-*xylo*-2-Hexulosonic Acid and Related Compounds in D_2O[a] and Me_2SO-d_6[b]

	1	2	3	4	5	6	-OCH_3
3a (D_2O)	172.2	96.9	73.3	74.3	70.0	63.4	
3b (D_2O)	170.5	96.6	72.8	73.7	69.5	62.8	53.9
3c[c] (D_2O)	64.5	98.5	71.4	74.8	70.3	62.7	
3d[d] (D_2O)	25.3	98.5	75.8	74.5	70.3	62.4	
3a (Me_2SO-d_6)	171.3	96.4	73.2	74.5	70.4	63.4	
3b (Me_2SO-d_6)	170.2	96.8	73.3	74.2	70.3	63.4	52.9
3c (Me_2SO-d_6)	64.4	97.8	71.0	74.6	70.4	62.4	

[a] Chemical shifts in parts per million from methanol standard assigned as 49.3 ppm relative to tetramethylsilane. [b] Chemical shifts in parts per million from Me_2SO-d_6 assigned at 39.6 ppm relative to tetramethylsilane. [c] Data taken from ref 9. [d] Data taken from ref 10.

Table II. ^{13}C Chemical Shifts of the Tautomers of Methyl D-*arabino*-2-Hexulosonate in D_2O[a] and Me_2SO-d_6[b]

	C_1	C_2	C_3	C_4	C_5	C_6	-OCH_3
6c (D_2O)	170.6	97.0	69.3	69.5	69.1	64.7	53.9
9c (D_2O)	170.6[c]	98.9	78.5	74.1	81.7	62.2	53.9[d]
8c (D_2O)	171.1[c]	103.8	83.5	75.8	83.0	61.6	53.6[d]
7c (D_2O)	170.6[c]	97.0	70.6[e]	71.7[e]	65.7[e]	63.5	53.5[d]
6c (Me_2SO-d_6)	170.2	97.2	69.6	69.8	69.1	63.0	52.5
9c (Me_2SO-d_6)	170.6[f]	99.2	79.2	75.0	82.8	64.6	52.5[g]
8c (Me_2SO-d_6)	170.2[f]	103.9	83.8	76.3	83.3	62.4	52.2[g]

[a] Chemical shifts in parts per million from methanol standard assigned as 49.3 ppm relative to tetramethylsilane. [b] Chemical shifts in parts per million from Me_2SO-d_6 standard assigned as 39.6 ppm relative to tetramethylsilane. [c-g] Assignments may be interchanged.

resonance decoupled spectra (SFORD) and from the spectra of specifically deuterated pure methyl L-[5-^{2}H]-*xylo*-hexulosonic acid (**3e**)[23] in Me_2SO-d_6.[24] The resonance at 70.3 ppm appears as a low-intensity triplet in C-5 deuterated **3e**. β-Deuterium isotope shifts of 0.034 and 0.05 ppm were observed for resonances at 74.2 and 63.4 ppm, respectively.[25-28] The SFORD spectra allowed assignment of these peaks to carbons at C-4 and C-6. The resonance at 73.3 ppm was not broadened on deuteration at C-5 and is thus assigned to C-3. In most of the spectra of **3a** and **3b** there is a small amount (<3%) of another tautomeric form which presumably is α-furanose **4a** or **4b**. However, definitive data were not obtained.

This result is in agreement with both the known solution conformation of L-sorbose (**3c**) in which the α-pyranose represents 98% of the tautomeric mixture with the other 2% being the α-furanose forms **5**[8,9] and the structure of L-*xylo*-2-hexulosonic acid in the crystalline state.[29] The relative chemical shift of the carbon atoms or tautomeric equilibrium of **3a** and **3b** was not *significantly* affected by the use of different solvents (D_2O, Me_2SO-d_6, pyridine-d_5, and DMF-d_7).

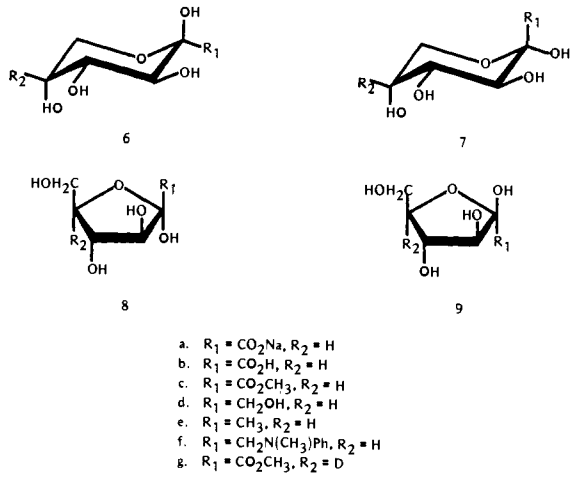
In Table I is shown a comparison of the chemical shifts of **3a, 3b, 3c,** and 1-deoxy-L-sorbose (**3d**). As can be seen, the chemical shift of C-4, C-5, and C-6 is not affected to any major degree by changing C-1 from -CO_2R to -CH_2OH to -CH_3. However, it should be noted that, in a comparison of **3a, 3b,** or **3c,** to **3d,** C-3 in **3d** is deshielded by 2.5-4.4 ppm. The original carbon assignments for L-sorbose (**3c**) made by Angyal and co-workers[10] and based on the expected β effect[27,28] at C-3 in **3d** (relative to **3c**) are consistent with our carbon assignments in **3a, 3b,** and **3c**.

Methyl D-*arabino*-2-Hexulosonate. The ^{13}C NMR spectrum of methyl D-*arabino*-2-hexulosonate in Me_2SO-d_6 surprisingly afforded a single tautomeric form to which was assigned structure **6c** (see Table II for the chemical shifts). However, in solution this material isomerized slowly to afford ultimately a mixture of four components, **6c, 7c, 8c,** and **9c.** The chemical shifts of **6c, 7c, 8c,** and **9c** were assigned based on SFORD and from the spectra of specifically deuterated methyl D-[5-^{2}H]-*arabino*-hexulosonic acid (**6g**).[30] The C-5 resonances of **6g, 8g,** and **9g** at 68.99, 83.22, and 82.75 ppm, respectively, appear as low-intensity triplets on substitution with deuterium. In addition, β-deuterium isotope shifts of 0.085 and 0.061 ppm at 69.8 and 63.0 ppm in a 1:1 mixture of **6c** and **6g** allowed the assignment of C-4 and C-6, respectively. Line broadening due to β-deuterium isotope effects was observed only at 76.3 and 75.0 ppm in 1:1 mixtures of tautomers **8c/8g** and **9c/9g,** respectively, allowing assignment of C-4 in these systems. Owing to the low concentration of pyranose tautomer **7c,** α- and β-deuterium isotope effects were not determined. The structural assignments of the furanose tautomers were made by comparison with the ^{13}C NMR spectrum of D-fructose and also are based on the well-documented fact[5-10] that the furanose **9c** with cis hydroxyl groups at C-2 and C-3 should have its anomeric carbon shielded relative to the anomeric carbon in **8c** in which these hydroxyl groups are trans. In addition thermodynamic predictions are in agreement with the relative order observed.[31,32] The tautomeric equilibrium at various times is summarized in Table III.

Table III. Tautomeric Mixture of Methyl D-*arabino*-2-Hexulosonate at Various Times (Me_2SO-d_6)

	time, days					
	0	1	5	12	19	27
6c	100	80	53	43	41	42[a]
9c		13	29	35	31	32
8c		7	18	22	28	27
7c		tr	tr	tr	tr	tr[a]

[a] Mixture of **6c** and **7c** in ratio of 6:1. The anomeric carbon of **7c** was not always clearly resolved from the anomeric carbon of **6c.** The amount of **7c** gradually increased from 0% at time zero to an equilibrium concentration of approximately 6%.



Not surprisingly, when a catalytic amount of *p*-toluenesulfonic acid was added to a sample of **6c** in Me_2SO-d_6, the tautomerization was *considerably faster.* These results are summarized in Table IV. Equilibrium appears to have been reached in less than 24 h in this case compared to more than 12 days in the uncatalyzed case. It must be noted that the anomeric carbons in **6c** and **7c** were not always adequately resolved to allow ratios of these two tautomers to be determined.

The fact that the initial solution spectrum of methyl D-*arabino*-2-hexulosonate in Me_2SO-d_6 showed exclusively the β-pyranose form provides strong evidence that this is the crystalline form of methyl D-*arabino*-2-hexulosonate.

Table IV. Acid-Catalyzed[a] Tautomeric Mixture of Methyl D-*arabino*-2-Hexulosonate at Various Times (Me_2SO-d_6)

	time, h					
	0.25	0.5	2.0	6.0	24.0	240
6c	72	57	53	40	39	39[b]
9c	16	20	27	31	35	34
8c	12	13	20	21	26	27
7c					tr	tr[b]

[a] *p*-Toluenesulfonic acid-H_2O. [b] The estimated ratio of **6c:7c** at equilibrium was 4:1 to 6:1 in these samples.

Table V. Composition of the Tautomeric Equilibrium of Methyl D-*arabino*-2-Hexulosonate in Various Solvents

	Me_2SO-d_6	D_2O	pyridine-d_5	DMF-d_7
6c	36[a]	67[b]	52	42
9c	32	15	29	39
8c	27	9	19	19
7c	6[a]	10[b]	*c*	*c*

[a] The chemical shifts of the anomeric carbons of **6c** and **7c** are very similar and not always resolved. The ratio given is approximate. [b] In most spectra the anomeric carbons of **6c** and **7c** were not resolved. [c] Pyranose tautomer **7c** was present in very low amounts.

While the tautomeric equilibrium is quite different in different solvents (Table V), only in Me_2SO-d_6 were the *tautomeric ratios significantly different initially than that observed in later spectra of the sample.* Clearly the pyranose tautomer is favored in D_2O while pyridine affords approximately a 1:1 mixture of pyranose:furanose forms, and Me_2SO and DMF favor slightly the furanose forms. This information clearly can be used to advantage in choosing solvents for specific chemical transformations.

Since it was observed that methyl D-*arabino*-2-hexulosonate slowly isomerized from the pure β-pyranose tautomer (**6c**) to an equilibrium mixture of **6c, 9c, 8c,** and **7c,** a closer look at the ^{13}C NMR spectrum of D-fructose in Me_2SO-d_6 was taken. It is well known that Me_2SO greatly slows down proton exchange and thus should greatly slow down the tautomeric equilibration of sugars as well. This effect has been referred to by Perlin et al.,[6] who observed that the tautomeric *equilibrium* of D-fructose in D_2O was maintained initially if the aqueous solution was concentrated and then dissolved in Me_2SO. On storage this *equilibrated to the equilibrium* present in Me_2SO. Doddrell and Allerhand[2] also reported that the ^{13}C NMR spectrum of D-fructose in D_2O originally afforded two components and on standing equilibrated to a four-component mixture. We were interested in determining whether or not the initial spectrum of D-fructose in Me_2SO-d_6 would show only the β-pyranose form (**6d**). Our initial attempts were made with D-fructose purchased from MCB (spray dried). When the spectrum was obtained, a tautomeric mixture had already formed (**6d:9d:8d:10** = 40:41:15:4). Since it is likely that the D-fructose used was not in one tautomeric form in the solid state but was already equilibrated, a sample was recrystallized from methanol. This afforded an initial spectrum showing a preponderance of the β-pyranose form (**6d:9d:8d** = 82:13:5). A more careful study revealed that the tautomeric equilibrium of fructose was established rapidly even in Me_2SO. These results are shown in Table VI. The rate at which this equilibrium is established as well as that for methyl D-*arabino*-hexulosonate will clearly be dependent on temperature, time, acid catalysis, and solvent purity. These results strongly suggest that in the crystalline state the D-fructose used was in the β-pyranose form. The crystal structures of D-fructose[33] and bis(β-D-fructopyranose)calcium chloride trihydrate[34] and dihydrate[35] all have been determined and all are in the β-pyranose form. It has been noted by Angyal[32] that all

Table VI. Tautomeric Equilibration of D-Fructose in Me_2SO-d_6

entry[b]	time, min	% **6d**	% **9d**	% **8d**	% **7d**[a]
1	8.8	81	10	9	
2	15.6	65	21	14	
3	22.4	58	33	9	
4	29.2	54	35	12	
5	103.8	43	46	11	
6	110.6	36	43	21	
7	117.4	37	45	19	
8	124.2	37	44	19	
9	24 h	31	45	19	5

[a] The percent of **6d** in entries 1-8 may include small amounts of **7d** as the anomeric carbons of **6d** and **7d** were not resolved in these spectra. [b] Each entry was a 6.8-min run, taken consecutively but not cumulatively from a starting time of 2 min after solvent addition.

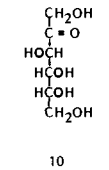
Table VII. ^{13}C Chemical Shifts of Tautomers of Sodium D-*arabino*-2-Hexulosonate in D_2O.[a]

	1	2	3[b]	4[b]	5[b]	6
6a	174.8	97.3	69.5	70.1	69.5	64.5
7a	*c*	*c*	71.2[d]	72.3[d]	67.0[d]	*c*
8a	*c*	104.2	82.9	75.8	82.4	61.4
9a	*c*	99.8	78.4	74.5	81.1	62.3

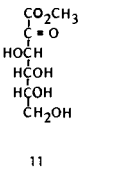
[a] Chemical shifts in parts per million standard assigned as 49.3 ppm related to tetramethylsilane. [b] The chemical shifts were assigned by analogy with deuterium-labeling studies on **6c, 9c, 8c,** and **7c** in Me_2SO-d_6. [c] Peaks not assigned. [d] Assignments may be interchanged.

pyranoid sugars studied by X-ray analysis have been found in the same chair conformations in the solid state as those that predominate in aqueous solution.

The much more rapid rate of tautomerization observed for D-fructose compared to methyl D-*arabino*-2-hexulosonate can be explained by comparing the energetics of the intermediates required for tautomerization to occur—namely, the open-chain keto form. The open-chain keto tautomer from D-fructose (**10**) is unexceptional. However, the open-chain keto tautomer from methyl D-*arabino*-2-hexulosonate generates an α-keto ester which contains an unfavorable electronic dipolar interaction. Therefore the rate of opening of tautomer **6c** to **11** would be predicted to be slower than the rate for the opening of **6d** to **10,** which agrees with the experimental data.



10



11

Finally the ^{13}C NMR spectrum of methyl D-[5-^2H]-*arabino*-2-hexulosonate was obtained in pyridine-d_5 (see Table X for a summary of the data). It was observed that for tautomers **6c, 9c,** and **8c** the resonances at 70.9, 84.6, and 85.5 ppm collapsed to broadened triplets. Since these were the same resonances assigned on the basis of the ^{13}C NMR spectrum of labeled tautomers **6c, 9c,** and **8c** in Me_2SO-d_6 this result supports the assumption that no changes occur in the ordering of the carbon chemical shifts on changes in solvent.[24]

Sodium D-*arabino*-2-Hexulosonate. The ^{13}C NMR spectrum of sodium D-*arabino*-2-hexulosonate in D_2O reveals the presence of four tautomeric forms. These are represented by **6a, 8a, 9a,** and **7a.** No open-chain keto acid was observed (<2%). The peaks were easily divided into four sets due to the differences in equilibrium concentrations of the four components. See Table VII for a summary of the data and the preceding discussion on methyl-D-*arabino*-2-hexulosonate for the method used in making the assignments. The tautomeric

Table VIII. Composition of the Tautomeric Equilibrium of D-*arabino*-2-Hexulosonic Acid in Various Solvents

	Me_2SO-d_6	D_2O	pyridine-d_5	DMF-d_7
6b	36	73	54	42
9b	35	16	28	36
8b	23	11	18	22
7b	6	tr	tr	tr

Table IX. ^{13}C Chemical Shifts of D-Fructose-Related Compounds in D_2O[a]

	1	2	3[d]	4[d]	5[d]	6
6a	174.8	97.3	69.5	70.1	69.5	64.5
6b	171.8	96.8	69.3	69.6	69.2	64.7
6c	170.6	97.0	69.3	69.5	69.1	64.7
6d[b]	64.7	99.1	68.4	70.5	70.0	64.1
6e[c]	25.6	98.9	72.9	70.4	70.0	64.1
9a		99.8	78.4	74.5	81.1	62.3
9b		98.7	78.5	74.2	81.6	62.1
9c		98.9	78.5	74.1	81.7	62.2
9d[b]	63.6	102.6	76.4	75.4	81.6	63.2
9e[c]	24.8	102.3	81.0	75.5	81.2	63.5
8a		104.2	82.9	75.8	82.4	61.4
8b		103.6	83.6	75.9	82.7	61.5
8c		103.8	83.5	75.8	83.0	61.6
8d[b]	63.8	105.5	82.9	77.0	82.2	61.9
8e[c]	22.8	105.9	83.1	77.3	82.0	62.0

[a] Chemical shifts in parts per million from methanol standard assigned as 49.3 ppm relative to tetramethylsilane. [b] Taken from ref 9 and confirmed in these laboratories. [c] Taken from ref 10. [d] The chemical shifts were assigned by analogy with deuterium-labeled derivatives of **6c**, **9c**, and **8c** (this work) and deuterated derivatives of D-fructose (ref 10).

equilibrium was determined to be approximately 80:trace:3:17 for **6a:7a:8a:9a** in D_2O.

D-*arabino*-2-Hexulosonic Acid. The ^{13}C NMR spectrum of D-*arabino*-2-hexulosonic acid[36] is as expected very similar to that of the corresponding sodium salt and methyl ester. In solution there are four tautomeric forms present—**6b, 9b, 8b,** and **7b.** In Table VIII is summarized the tautomeric equilibria of D-*arabino*-2-hexulosonic acid in various solvents. In D_2O the pyranose form (**6b**) is favored, while in the more polar solvents DMF and Me_2SO the furanose forms (**9b** and **8b**) are favored. The tautomeric equilibrium does not appear to be affected substantially whether or not the free carboxylic acid, sodium salt, or methyl ester is present at C-1. The solvent dependence of the pyranose to furanose forms has been previously reported.[6]

In Table IX is shown a comparison of the chemical shifts of a variety of D-fructose-related materials. As can be seen, within each series of compounds little effect on the chemical shift of the carbons is observed at C-4, C-5, and C-6 when C-1 is varied from $-CH_3$ to $-CH_2OH$ to $-CO_2R$. The β effect[27,28] resulting from changes at C-1 causes C-3 to vary from approximately -0.5 to $+4.6$ ppm in the tautomers of each series.

Funcke and Klemer[11] have recently reported the ^{13}C NMR spectra for D-fructose and a number of 1-amino-1-deoxy-D-fructose derivatives in pyridine-d_5. Within this series, the tautomeric equilibrium did not vary greatly and the chemical shift of the atoms in each tautomeric form did not vary greatly with different amine substituents. In Table X several sets of data from the spectra of these compounds are compared with that from **6b, 6c, 8b, 8c,** and **9b,c.** Again major chemical shift differences are confined to C-1, C-2, and C-3. In C-4 to C-6, only minor changes appear to be present.

Sodium D-*xylo*-5-Hexulosonate. The ^{13}C NMR spectrum of sodium D-*xylo*-5-hexulosonate in D_2O shows the presence of three major components. One is the open-chain keto form

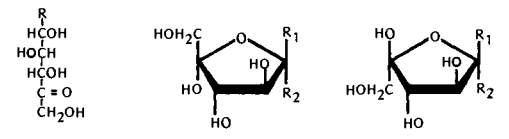
Table X. ^{13}C Chemical Shifts of D-Fructose-Related Compounds in Pyridine-d_5[a]

	1	2	3[b]	4[b]	5[b]	6
6b	173.7	98.5	71.4	71.9	70.8	65.6
6c	171.6	99.0	71.6	71.8	70.9	65.8
6d[d]	67.1	100.6	71.0	72.6	71.6	65.8
6f[d]	59.9	100.5	71.0[c]	71.8[c]	70.6[c]	64.3
9b	174.2	100.9	81.2	76.9	84.3	64.3
9c	172.0	101.1	81.4	76.8	84.6	64.4
9d[d]	65.1	105.0	79.1	78.0	84.7	64.7
9f[d]	58.5	104.4	79.1[c]	76.6[c]	83.6[c]	63.6
8b	174.4	105.1	85.3	76.9	84.7	63.1
8c	171.9	105.9	85.7	77.9	85.5	63.6
8d[d]	66.2	107.5	85.3	79.1	84.7	63.3
8f[d]	57.5	107.8	82.6[c]	80.0[c]	85.7[c]	62.8

[a] Chemical shifts in parts per million relative to dioxane assigned as 67.4 ppm relative to tetramethylsilane. [b] Shifts assigned by analogy with deuterium-labeled derivatives of **6c, 8c,** and **9c** (this work) and deuterium-labeled derivative of D-fructose (ref 10). [c] Assignments may be interchanged. [d] Data taken from ref 11 and assignments from ref 10.

12a and the other two are furanose forms **13a** and **14a.** The major furanose form was assigned to **13a** using arguments similar to those used for determining the structures of the two furanoses present in a solution containing methyl D-*arabino*-2-hexulosonate. It must be noted that the crystal structure of calcium D-*xylo*-5-hexulosonate dihydrate has been determined[37] and corresponds to the major furanose form (**13a**) in aqueous solution. These data are in agreement with the conclusions reached by Chen and co-workers,[38] who studied the tautomers of D-*xylo*-5-hexulosonate in water by UV, IR, and ^1H NMR. Our observed tautomeric equilibrium of 79:10:11 in D_2O for **13a:14a:12a** is remarkably similar to the tautomeric equilibrium reported for the corresponding tautomers of D-*threo*-2-pentulose:[10] **13b:14b:12b** = 62:16:22.

The ^{13}C NMR data for **12a, 13a,** and **14a** are summarized in Table XI. Carbon chemical shift assignments are based on



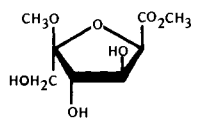
12

a. R = CO_2Na
b. R = H

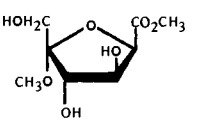
13

a. R_1 = CO_2Na, R_2 = H
b. R_1 = H, R_2 = H
c. R_1 = H, R_2 = CO_2Na

14



15



16

chemical shifts, SFORD spectra, and a comparison with the chemical shifts for the known furanose tautomers of D-*threo*-2-pentulose.[10] The assignment of C-2 in **13a** was obtained from the specifically deuterated D-[2-^2H]-*xylo*-5-hexulosonic acid.[30] Thus the results obtained for sodium D-*xylo*-5-hexulosonate are consistent with the results previously reported for D-*threo*-pentulose.

When D-*xylo*-5-hexulosonic acid was treated with methanol and *p*-toluenesulfonic acid, a mixture of two furanosides was obtained.[39] Structures **15** and **16** were assigned to these compounds based on the ^{13}C NMR data which are summarized in Table XI.

Sodium D-*lyxo*-5-Hexulosonate. Only the two furanose tautomers **13c** and **14c** are observed in the ^{13}C NMR spectrum

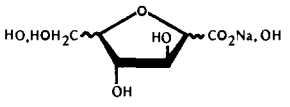
Table XI. ^{13}C Chemical Shifts of the Tautomers of Sodium D-*xylo*-5-Hexulosonate, Sodium D-*lyxo*-5-Hexulosonate, and Related Compounds in D_2O[a]

	1	2	3	4	5	6
13a	176.5	80.1	77.1[b]	77.0[b]	103.8	64.5
14a	176.9	83.9[c]	80.4[c]	77.6[c]	107.6	63.3
12a	178.8	[d,e]	73.0[e]	73.8[e]	213.4	66.9
13b[f,g]		70.6	75.7	77.1	103.7	63.9
14b[f,g]		73.7	76.7	81.4	106.5	63.3
12b[f,g]		62.5	72.7	76.1	213.4	66.9
13c	178.0	80.6	78.2[h]	76.1[h]	102.7	63.2
14c	177.5	81.8	81.5[i]	79.8[i]	105.5	62.7
15[l]	171.3	82.1[j]	78.1[j]	77.2[j]	110.9	56.4
16[m]	171.4	76.2[k]	77.8[k]	77.5[k]	104.7	60.1

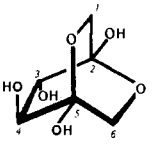
[a] Chemical shifts in parts per million relative to methanol internal standard assigned as 49.3 ppm relative to tetramethylsilane. [b,c,e,h,i,j,k] Assignments may be interchanged. [d] Assignment was not made. [f] Data taken from ref 10. [g] For comparative purposes the numbering used for D-*threo*-2-pentulose tautomers (**13b, 14b,** and **12b**) is that shown in the table. [l] The ester methyl is at 52.4 ppm; the ketal methyl is at 49.0 ppm. [m] The ester methyl is at 52.5 ppm; the ketal methyl is at 48.8 ppm.

of sodium D-*lyxo*-5-hexulosonate in a ratio of 72:28, respectively. The major furanose form was assigned to **13c** using arguments similar to those previously presented. Carbon assignments were based on the chemical shift, SFORD spectra, and specifically deuterated D-[2-^{2}H]-*lyxo*-5-hexulosonic acid[30] and are similar to the shifts assigned to **13a** and **14a** (see Table XI).

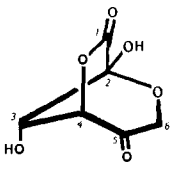
Calcium D-*threo*-2,5-Hexodiulosonate. Since the chemical structure of calcium D-*threo*-2,5-hexodiulosonate was determined by Katznelson and co-workers,[40] several tautomers have been proposed for its solution structure. On the basis of polarographic data, Bernaerts and De Ley[41] suggested that tautomers **17** might represent the solution structure for calcium D-*threo*-2,5-hexodiulosonate. By analogy with the proposed structure for D-*threo*-2,5-hexodiulose (**18**),[42] Imada and Asano[43] suggested that tautomer **19** might represent the solution structure for calcium D-*threo*-2,5-hexodiulosonate. We have obtained ^{13}C NMR data from calcium D-*threo*-2,5-hexodiulosonate which are not consistent with either tautomer **17** or **19,** but rather support tautomer **20** as the major tautomer



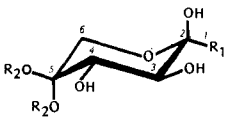
17



18



19



20

a. $R_1 = CO_2Ca_{1/2}$, $R_2 = H$
b. $R_1 = CO_2CH_3$, $R_2 = H$
c. $R_1 = CO_2CH_3$, $R_2 = CH_3$
d. $R_1 = CH_2OH$, $R_2 = H$
e. $R_1 = CH_3$, $R_2 = H$

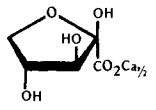
for freshly prepared material in aqueous solution.[21] There is little or no keto component present. The chemical shifts are shown in Table XII. Additional support for this assignment was obtained when the ^{13}C NMR of calcium D-*threo*-2-pentulosonate was obtained. This material clearly can exist

Table XII. ^{13}C Chemical Shifts for D-*threo*-Hexodiulose Related Compounds and Calcium D-*threo*-2-Pentulosonate Tautomers (D_2O)[c]

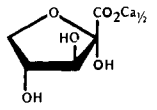
	1	2[b]	3[a]	4[a]	5[b]	6
20a	175.4	93.6	72.2	74.3	97.7	66.7
20b	170.5	92.9	71.6	73.3	97.0	66.1
20c[d]	169.9	96.6	71.9	74.2	97.7	60.4
20d	65.4[e]	93.1	70.0	73.7	98.3	64.2[e]
21	175.8	101.6	79.0	75.1	71.2	
22	174.7	105.6	81.6	75.5	73.2	

[a] Assignments may be interchanged. [b] Assignments for carbons 2 and 5 based on D_2O/H_2O exchange experiments on **20c.** [c] Chemical shifts in parts per million from methanol standard assigned as 49.3 ppm relative to tetramethylsilane. [d] Chemical shifts in parts per million from Me_2SO-d_6 assigned as 39.6 ppm relative to tetramethylsilane. Solvent was Me_2SO-d_6. [e] Assignments may be interchanged.

only in two furanose tautomers and an open-chain tautomer. The ^{13}C NMR data are consistent with the presence of furanose tautomers **21** and **22.** Little if any open-chain keto tau-



21



22

tomer was present. These data are summarized in Table XII.

The methyl ester dimethoxy ketal **20c** also exists predominantly in a single pyranose tautomer, and has been shown to exist in this conformation in the crystal state as well.[44] By analogy it has been concluded that freshly isolated D-*threo*-2,5-hexodiulose (**20d**) exists predominantly as a pyranose tautomer with a hydrated 5-keto group rather than structure **18** as previously proposed.[42]

The chemical shifts of the anomeric carbons at C-2 and C-5 in **20a–e** are consistent with a pyranose conformation, with the anomeric carbon resonances in **21** and **22** being deshielded by 3–9 ppm relative to any anomeric carbon in **20a–e.** In addition the chemical shifts of C-3 and C-4 in **20a–e** are more consistent with a pyranose ring tautomer (compare with the chemical shifts of C-2 and C-3 in **21** and **22**). The recent characterization[45] of 6-deoxy-D-*threo*-2,5-hexodiulose and its methyl pyranoside dimethoxy ketal (**20e**) as a pyranose tautomer based on ^1H NMR is also in agreement with our assignments in the 2,5-hexodiulose series.

Carbon assignments for **20a–e** are based on chemical shift and SFORD spectra for carbons C-1 and C-6. Anomeric carbons C-2 and C-6 are based on H_2O/D_2O deuterium isotope shift experiments on **20c** in Me_2SO-d_6.[46,47] A 35% increase observed in the signal width at half-height of the carbon resonance at 96.6 ppm relative to the absorption at 97.6 ppm suggests that the upfield quaternary carbon is appended with the anomeric hydroxyl moiety. Unequivocal assignment of C-3 and C-4 is not possible at this time.

Experimental Section

The ^{13}C NMR spectra were *obtained* on a Varian XL-100A-15 (25.2 MHz) spectrometer equipped with a Nicolet Technology 1080 data system. Proton decoupling was provided by square wave modulation[48] of the Varian gyrocode heteronuclear decoupler. The spectra were obtained with a tip angle of approximately 30°, an acquisition time of 1.4 s using quadrature phase detection, and computer resolution of 1.4 Hz (sweep width 6 kHz; 8K data points). The field frequency lock was maintained by the solvent deuterium resonance in a 5-mm sample tube (o.d.) and the internal standard adjusted to provide chemical-shift values relative to tetramethylsilane. Standards and shifts (ppm) used were Me_2SO-d_6 (39.6), dioxane (67.4),

methanol (49.3), and $CDCl_3$ (76.9).[49] All spectra were recorded at ambient probe temperature.

The extent of the β-carbon deuterium isotope effects was determined by examination of a 1:1 mixture of deuterated and undeuterated material[25,27,28] in Me_2SO-d_6 solvent.[24] Separation of partially resolved peaks was determined by deconvolution using the curve analysis program of J. W. Akett provided by the Nicolet Users Society. Carbon-atom assignments were made by off-resonance decoupling, chemical shift, and deuterium isotope substitution. The identity of the different tautomers was determined by comparison with literature precedent for related keto sugars[8-10] and by the use of the cis effect on the anomeric carbon in furanose rings.[5-10] The ratio of tautomeric components in solution was determined by the peak heights of the anomeric carbons. To check the accuracy of this method, T_1's for the anomeric carbons, measured by progressive saturation, were shown to be 5.82 and 6.47 s for a mixture of **6c** and **3c** in water, respectively, corresponding to a calculated relative error of about 5%. Variation of the pulse tip angles from 10 to 30° on **6c** and **3c** also showed no change in relative peak heights for the anomeric carbons. In addition T_1's for the anomeric carbons in **6c, 9c,** and **8c** were shown to be 4.22, 5.28, and 4.46 s, respectively, in Me_2SO-d_6. Based on these data it was assumed that the height of the various anomeric carbon atoms in each sample accurately reflected the ratio of tautomers present.

The compounds used were prepared according to procedures described in the appended references. These were sodium D-*arabino*-2-hexulosonate,[50-52] D-*arabino*-2-hexulosonic acid,[36] methyl D-*arabino*-2-hexulosonate,[50,51] sodium L-*xylo*-2-hexulosonate,[15] methyl L-*xylo*-2-hexulosonate,[15] L-*xylo*-2-hexulosonic acid,[15] calcium D-*xylo*-5-hexulosonate,[50,53] methyl (methyl D-*threo*-5-hexulofuranosid)onate,[39] calcium D-*threo*-2,5-hexodiulosonate,[54,55] D-*threo*-2,5-hexodiulose,[56] calcium D-*threo*-2-pentulosonate,[57,58] D-[5-^{2}H]-*arabino*-2-hexulosonic acid and methyl ester, L-[5-^{2}H]-*xylo*-2-hexulosonic acid[23] and methyl ester, D-[2-^{2}H]-*xylo*-5-hexulosonate,[30] calcium D-*lyxo*-5-hexulosonate,[59] and sodium D-[2-^{2}H]-*lyxo*-5-hexulosonate.[30]

D-Fructose was recrystallized by dissolving 10 g in hot methanol, concentrating to approximately 60 mL, and allowing to slowly cool to room temperature. After standing at room temperature for 2 days the large, transparent crystals were collected and dried (0.54 g, mp 111–120 °C).

Calcium D-*xylo*-5-hexulosonate and calcium D-*lyxo*-5-hexulosonate, which are only sparingly soluble in water,[60] were converted to their soluble sodium salts either by adjusting the pH of an aqueous heterogeneous solution containing the calcium salt to 2 with sulfuric acid, filtering off the calcium sulfate, adding sodium hydroxide, and crystallizing or stirring the calcium salt with an excess of Dowex 50W-X8 cation exchange resin in the sodium form and crystallizing.

The crystalline nature of D-*arabino*-2-hexulosonic acid[36] was demonstrated by its X-ray powder diffraction pattern. However, this does not provide any evidence as to whether or not the crystals consist of a single tautomeric form or a mixture of tautomeric forms.

Acknowledgments. The authors would like to thank Dr. D. A. Kita for providing calcium D-*threo*-2,5-hexodiulosonate, calcium D-*threo*-2-pentulosonate, and calcium D-*xylo*-5-hexulosonate, Mr. J. D. Gillis for providing sodium D-*arabino*-2-hexulosonate and the corresponding methyl ester and sodium L-*xylo*-2-hexulosonate and the corresponding free acid and methyl ester, and Mr. J. Donnelly and Mr. D. Holstein of the Minerals, Pigments, and Metals Division of Pfizer Inc. for the X-ray powder pattern on D-*arabino*-2-hexulosonic acid. In addition we would like to thank Drs. E. B. Whipple and D. Horton for valuable discussions during the course of this work.

References and Notes

(1) Dorman, D. E.; Roberts, J. D. *J. Am. Chem. Soc.* **1971,** *93,* 4463–4472.
(2) Doddrell, D.; Allerhand, A. *J. Am. Chem. Soc.* **1971,** *93,* 2779–2781.
(3) Koerner, T. A. W.; Cary, L. W.; Bhacca, N. S.; Younathan, E. S. *Biochem. Biophys. Res. Commun.* **1973,** *51,* 543–550.
(4) Gray, G. R. *Acc. Chem. Res.* **1976,** *11,* 418–424, and references cited therein.
(5) Perlin, A. S.; Cyr, N.; Koch, H. J.; Korsch, B. *Ann. N.Y. Acad. Sci.* **1973,** *222,* 935–942.
(6) Perlin, A. S.; DuPenhoat, P. H.; Isbell, H. S. *Adv. Chem. Ser.* **1973,** *117,* 39–50.
(7) Ritchie, R. G. S.; Cyr, N.; Korsch, B; Koch, H. J.; Perlin, A. S. *Can. J. Chem.* **1975,** *53,* 1424–1433.
(8) Que, L., Jr.; Gray, G. R. *Biochemistry* **1974,** *13,* 146–153.
(9) Angyal, S. J.; Bethell, G. S. *Aust. J. Chem.* **1976,** *29,* 1249–1265.
(10) Angyal, S. J.; Bethell, G. S.; Cowley, D. E.; Pickles, V. A. *Aust. J. Chem.* **1976,** *29,* 1239–1247.
(11) Funcke, W.; Klemer, A. *Carbohydr. Res.* **1976,** *50,* 9–13.
(12) Hyvonen, L.; Varo, P.; Kolvistoinen, P. *J. Food Sci.* **1977,** *42,* 657–659.
(13) Ohle, H. *Angew. Chem.* **1933,** *46,* 399–400.
(14) Mauer, K.; Schiedt, B. *Ber.* **1933,** *66,* 1054–1057.
(15) Reichstein, T.; Grüssner, A. *Helv. Chim. Acta* **1934,** *17,* 311–328.
(16) Chen, C.; Yamamoto, H.; Kwan, T. *Chem. Pharm. Bull.* **1970,** *18,* 1305–1309.
(17) Sonoyama, T.; Kageyama, B.; Honjo, T. U.S. Patent 3 922 194, 1975.
(18) Sonoyama, T.; Tani, H.; Kagenyama, B.; Kobayashi, K.; Honjo, T.; Yagi, S. U.S. Patent 3 963 574, 1976.
(19) Sonoyama, T.; Tani, H.; Kageyama, B.; Kobayashi, K.; Honjo, T.; Yagi, S. U.S. Patent 3 959 076, 1976.
(20) Sonoyama, T.; Tani, H.; Kageyama, B.; Kobayashi, K.; Honjo, T.; Yagi, S. U.S. Patent 3 998 697, 1976.
(21) Andrews, G.; Bacon, B.; Crawford, T.; Breitenbach, R. *J. Chem. Soc., Chem. Commun.* **1979,** 740–741. Andrews, G. C. U.S. Patent 4 159 990, 1979.
(22) Loewus, F. *Annu. Rev. Plant Physiol.* **1971,** *22,* 337–364, and references cited therein.
(23) Ogawa et al. (Ogawa, T.; Taguchi, K.; Takasaka, N.; Mikata, M.; Matsui, M. *Carbohydr. Res.* **1976,** *51,* C1–C4) recently reported the preparation of a derivative of methyl L-[5-^{2}H]-*xylo*-hexulosonate. We will report the details of our unambiguous synthesis of [5-^{2}H]-**3b** in due course.
(24) It has been assumed in the extrapolation of the deuterium-labeling results obtained in Me_2SO-d_6 to spectra taken in other solvents that no changes occur in the ordering of the carbon chemical shifts.
(25) Wehrli, F. W.; Wirthlin, T. "Interpretation of Carbon-13 NMR Spectra"; Heyden & Sons: New York, 1976; pp 108–109, and references cited therein.
(26) See the Experimental Section for Methods of obtaining spectrum.
(27) Gorin, P. A. J. *Can. J. Chem.* **1974,** *52,* 458–461.
(28) Gorin, P. A. J.; Mazurek, M. *Can. J. Chem.* **1975,** *53,* 1212–1223.
(29) Hvoslef, J.; Bergen, B. *Acta Crystallogr., Sect. B* **1975,** *31,* 697–702.
(30) The methods for the preparation of D-[5-^{2}H]-*arabino*-2-hexulosonic acid (**6g**), D-[2-^{2}H]-*xylo*-5-hexulosonic acid, and D-[2-^{2}H]-*lyxo*-5-hexulosonic acid will be reported in subsequent publications.
(31) Angyal, S. J. *Aust. J. Chem.* **1968,** *21,* 2737–2746.
(32) Angyal, S. J. *Angew. Chem., Int. Ed. Engl.* **1969,** *8,* 157–166.
(33) Rosenstein, R. D. Abstracts, American Crystallographic Association Meeting, Buffalo, N.Y., Aug. 1968, No. 92. Taken from *Adv. Carbohydr. Chem. Biochem.* **1975,** *31,* 365. To our knowledge no details have yet been published. Also see: *Adv. Carbohydr. Chem. Biochem.* **1975,** *24,* 60, 103.
(34) Craig, D. C.; Stephenson, N. C.; Stevens, J. D. *Cryst. Struct. Commun.* **1974,** *3,* 195–199.
(35) Craig, D. C.; Stephenson, N. C.; Stevens, J. D. *Cryst. Struct. Commun.* **1974,** *3,* 277–281.
(36) Faubl, H.; Kolb, C. A. *Carbohydr. Res.* **1978,** *63,* 315–317.
(37) Balchin, A. A.; Carlisle, C. H. *Acta Crystallogr.* **1965,** *19,* 103.
(38) Chen, C.; Yamamoto, H.; Kwan, T. *Chem. Pharm. Bull.* **1970,** *18,* 815–819.
(39) Jones, J. K. N.; Reid, W. W. *Can. J. Chem.* **1955,** *33,* 1682–1683.
(40) Katznelson, H.; Tanenbaum, S. W.; Tatum, E. L. *J. Biol. Chem.* **1953,** *204,* 43–59.
(41) Bernaerts, M.; De Ley, J. *Antonie van Leeuwenhoek* **1971,** *37,* 185–195.
(42) Englard, S.; Avigad, G.; Prosky, L. *J. Biol. Chem.* **1965,** *240,* 2302–2307.
(43) Imada, K.; Asano, K. *Chem. Pharm. Bull.* **1974,** *22,* 1691–1698.
(44) Andrews, G. C.; Bacon, B.; Bordner, J.; Hennessee, G. L. A. *Carbohydr. Res.* **1979,** *77,* 25–36.
(45) Dizdaroglee, M.; Leitich, J.; von Sonntag, C. *Carbohydr. Res.* **1976,** *47,* 15–23.
(46) Ho, S.; Koch, H. J.; Stuart, R. S. *Carbohydr. Res.* **1978,** *64,* 251–256.
(47) Gagnaire, D.; Vincendon, M. *J. Chem. Soc., Chem. Commun.* **1977,** 509–510.
(48) Grutzner, J. B.; Santini, R. E. *J. Magn. Reson.* **1975,** *19,* 173–187.
(49) Levy, G. C.; Nelson, G. L. "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists"; Wiley-Interscience: New York, 1972.
(50) Stubbs, J. J.; Lockwood, L. B.; Roe, E. T.; Tabenkin, B.; Ward, G. E. *Ind. Eng. Chem.* **1940,** *32,* 1626–1631.
(51) Lockwood, L. B.; Tabenkin, B.; Ward, G. E. *J. Bacteriol.* **1941,** *42,* 51–61.
(52) Lockwood, L. B. *Methods Carbohydr. Chem.* **1963,** *2,* 51–54.
(53) Lockwood, L. B. *Methods Carbohydr. Chem.* **1963,** *2,* 54–56.
(54) Oga, S.; Sato, K.; Imada, K.; Asano, K. U.S. Patent 3 790 443, 1974.
(55) A convenient method for the preparation of analytically pure samples of calcium D-*threo*-2,5-hexodiulosonate will be reported in a subsequent publication.
(56) Terada, O.; Tomizawa, K.; Suzuki, S.; Kinoshita, S. *J. Agric. Chem. Soc. Jpn.* **1961,** *35,* 131–134.
(57) Takahashi, T.; Murakawa, S.; Barada, M. *Agric. Biol. Chem.* **1976,** *40,* 1255–1256.
(58) Yasuda, N. Japanese Patent 43, 28 950 (1968); *Chem. Abstr.* **1969,** *70,* 95465n.
(59) Nelson, E. R.; Nelson, P. F. *Aust. J. Chem.* **1968,** *21,* 2323–2325.
(60) Teramoto, S.; Hori, I. *J. Ferment. Technol.* **1948,** *26,* 7–9.