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Note

Detection of the open-chain forms of D-fructose and L-sorbose in aqueous solution by using ^{13}C-n.m.r. spectroscopy

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The mutarotation equilibria of aldoses and ketoses in solution have been investigated by using polarimetry[1], o.r.d., c.d., u.v. spectroscopy[2–6], ^{1}H-n.m.r.[6–12] and ^{13}C-n.m.r. spectroscopy[12–18], and g.l.c.–m.s.[19,20].

The interpretation of data is often difficult and the results obtained by different methods are not consistent. ^{1}H-N.m.r. spectroscopy of ketoses is more difficult than that of aldoses, and it was possible to identify only two of the major isomers of D-fructose[11]. ^{13}C-N.m.r. spectroscopy revealed the four cyclic isomers of D-fructose in aqueous solution[13]. Subsequently, extensive studies of several hexuloses were pubblished[14–17]. The open-chain forms of ketoses were observed only by ^{13}C-n.m.r. spectroscopy for solutions of 1-deoxyhexuloses in water[21] and D-fructose in pyridine[18].

The presence of the open-chain form of D-fructose in aqueous solution has been indicated by g.l.c.–m.s.[19,20], u.v. spectroscopy[2], and c.d.[6]. Moreover, the products[22] of the u.v. photolysis of D-fructose in aqueous solution at 254 nm are well accounted for if they arise from the excitation of the open-chain form (*i.e.*, α-cleavage of the carbonyl chromophore), and are different from those expected from acetals[23]. Furthermore, at this wavelength, the acetal chromophore is not expected to show significant absorption[23]. This behaviour posed the question of the proportion of D-fructose in the open-chain form at mutarotation equilibrium in aqueous solution.

We now report on the application of ^{13}C-n.m.r. spectroscopy to this problem and also to L-sorbose.

A ^{13}C-n.m.r. spectrum having 21 signals belonging to four isomers (α- and β-furanose, and α- and β-pyranose) has been reported[17] for an aqueous solution of D-fructose at equilibrium; the four isomers are present in very different amounts (Table I). Signal assignments were based[17] on hydrogen/deuterium exchange, but the open-chain form was not detected.

TABLE I

^{13}C-CHEMICAL SHIFTS[a] FOR SOLUTIONS OF D-FRUCTOSE AND L-SORBOSE IN DEUTERIUM OXIDE

Isomer	*Atom*	D-*Fructose*[b]	D-*Fructose*[17]	1-*Deoxy*-D-*fructose*[21]	L-*Sorbose*[b]	L-*Sorbose*[17]	1-*Deoxy*-L-*sorbose*[21]
	C-1	64.5	63.8	22.8	64.6	64.3	26.6
	C-2	105.7	105.5	105.9	102.6	102.5	n.s.[c]
	C-3	83.4	82.9	83.1	77.5	77.0	81.4
α-f	C-4	77.9	77.0	77.3	76.6	76.2	76.2
	C-5	83.0	82.2	82.0	78.6	78.6	78.3
	C-6	62.7	61.9	62.0	61.4	61.6	61.5
	C-1	64.7	63.6	24.8	63.6		
	C-2	102.8	102.6	102.3	n.s.[c]		
	C-3	77.5	76.4	81.0	82.0[d]		
β-f	C-4	76.3	75.4	75.5	81.5[d]		
	C-5	82.1	81.6	81.2	77.0[d]		
	C-6	63.7	63.2	63.5	61.8		
	C-1	63.2[d]	65.9[d]	19.4	64.8	64.5	25.3
	C-2	99.0	n.s.[c]	100.0	98.3	98.5	98.5
	C-3	71.8[d]	70.9	73.7	71.7	71.4	75.8
α-p	C-4	72.1[d]	71.3	71.8	74.8	74.8	74.5
	C-5	66.2	n.s.[c]	67.7	70.2	70.3	70.0
	C-6	62.2[d]	n.s.[c]	62.7	62.7	62.7	62.4
	C-1	65.6	64.2	25.6	63.8[d]		
	C-2	99.1	99.1	98.9	n.s.[c]		
	C-3	69.3	68.4	72.9	72.3[d]		
β-p	C-4	71.1	70.5	70.4	74.1[d]		
	C-5	70.4	70.0	70.0	69.9[d]		
	C-6	64.6	64.1	64.1	63.3[d]		
	C-1	67.3		26.7	66.8		26.4
	C-2	214.2		213.9	n.s.[c]		n.s.[c]
Open-	C-3	n.s.[c]		77.7	n.s.[c]		78.3
chain	C-4	72.8[d]		71.7	73.3[d]		72.9
	C-5	71.5[d]		71.5	72.6[d]		71.8
	C-6	64.2[d]		63.7	n.s.[c]		63.2

[a]P.p.m. downfield from tetramethylsilane. [b]Measurements at 80°. [c]Signals not seen (not separated from major signals, or of too low intensity because of long relaxation time). [d]Uncertain assignments.

The open-chain form can be readily recognised (Table I) by increasing the concentration of D-fructose (3.7M instead of 0.5–1.5M) and the resonance frequency of ^{13}C (67.89 instead of 22.63 MHz). An increase in the temperature is also helpful, as it has been shown that the proportion of the open-chain form of 1-deoxyhexuloses is thereby enhanced[21].

Of the expected 30 ^{13}C-signals for D-fructose, 29 are observed at 80°. Most of them can be assigned on the basis of the data of Angyal *et al.*[17,21] for D-fructose and 1-deoxy-D-fructose (Table I). As expected, the carbonyl group of the open-chain form appears at δ 214. Apart from that for C-1, the other signals for this form are

similar to those for 1-deoxy-D-fructose. Only the C-3 signal is not seen, possibly because it is hidden below the more intense C-3 or C-4 signals of the β-furanose; the C-3 signal of the open-chain form of 1-deoxy-D-fructose occurs at δ 77.7.

Hitherto, only the signals for two isomers (α-pyranose and α-furanose[14,17]) have been detected in ^{13}C-n.m.r. spectra of L-sorbose. Although L-sorbose is less soluble in water than D-fructose, we observed 25 signals belonging to five different isomers. Those of the main isomers (α-pyranose and α-furanose) are readily assigned by comparison with previous data[17]. The positions of the ^{13}C signals of the open-chain form are similar to those of 1-deoxy-L-sorbose[21], apart from that of C-1 (Table I). The C-3,4,5 signals of the β-furanose are very similar to those[17] of methyl β-L-sorbofuranoside. As usual, the β-furanose signals appear at a lower field than those of the β-pyranose[24].

In order to quantify the ratios of the five isomers of D-fructose and L-sorbose present in aqueous solution at 80°, the integrals of the signals for C-1,6 and C-3,4,5 were averaged. Both sets of data gave the same results within the experimental error (*cf.* Ref. 17). The C-2 signals were not considered, because of the different relaxation times of the anomeric and the carbonyl carbons. As can be seen from Table II, our data accord with, and supplement, those reported previously for D-fructose and L-sorbose. An increase in the concentration of the open-chain form with increase in temperature is also evident from the u.v. absorption at 280 nm ($n \rightarrow \pi^*$ transition of the C=O chromophore). The intensity of this absorption for an aqueous solution of D-fructose increases by a factor of 3 to 4 as the temperature increases from 20° to 80°. At 80°, slight decomposition of D-fructose occurs, generating absorptions at 280 and ~225 nm. The decomposition product could not be detected by ^{13}C-n.m.r. spectroscopy and, hence, must have a high u.v. extinction coefficient. Similar, but more extensive, decomposition was observed for L-sorbose, and this prevented quantification of the open-chain form at room temperature.

TABLE II

PERCENTAGE OF THE ISOMERS OF D-FRUCTOSE AND L-SORBOSE AT EQUILIBRIUM FOR SOLUTIONS IN DEUTERIUM OXIDE

	Temp. (degrees)	*Isomer*					*Ref.*
		α-f	β-f	α-p	β-p	*Open-chain*	
D-Fructose	27	4	21	trace	75		17
	55	6	28	trace	66		17
	85	11	33	trace	56		17
	80	10	32	2	53	3	this study
L-Sorbose	27	2		98			17
	55	4		96			17
	85	9		91			17
	80	8	1	87	2	2	this study

The u.v. measurements indicate that, at room temperature, ~0.8% of the open-chain form of D-fructose is present (*cf.* 0.7% obtained[6] by c.d.). The apparent molar extinction coefficient (ε 0.45M^{-1}.cm^{-1}) at 280 nm for 0.1–3M D-fructose was independent of concentration, *i.e.*, there is no change in the relative concentration of the open-chain form. Using the value of ε and a concentration of the open-chain form of 0.8%, a molar extinction coefficient of ~56M^{-1}.cm^{-1} was obtained for the carbonyl n→π* transition [*cf.* ε (267 nm) 20M^{-1}.cm^{-1} for hydroxyacetone[25]].

EXPERIMENTAL

D-Fructose and L-sorbose were commercial samples and were used without further purification. Proton-decoupled, ^{13}C-n.m.r. spectra were recorded at 67.8 Hz with a Bruker WH 270 instrument (internal deuterium lock and operation in the Fourier-transform mode). The instrument was equipped with a Nicolet BNC 12 Computer having a 32 K data memory and a Diablo Disk-System. All the measurements in Table I were performed at 80° on 3.7M D-fructose and 2.1M L-sorbose in deuterium oxide in 10-mm sample-tubes with a spinning rate of ~30 sec^{-1}. For D-fructose, 56000 scans with a pulse sequence of 1 sec, and for L-sorbose, 68000 scans with a pulse sequence of 0.6 sec were used to record the spectra. The internal standard was the Me_2SO signal at δ 40.6 (relative to that of Me_4Si).

U.v. spectra were recorded for equilibrated, aqueous solutions with a Brückel HRS 4001 C instrument. Identical results were obtained after elimination of air by several freeze-pump cycles.

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