α-D-Glucopyranosyl-D-fructoses: Distribution of Furanoid and Pyranoid Tautomers in Water, Dimethyl Sulphoxide, and Pyridine. Studies on Ketoses. Part 4[1]

Frieder W. Lichtenthaler* and Stephan Rönninger
Institut für Organische Chemie, Technische Hochschule Darmstadt, Petersenstraße 22, D-6100 Darmstadt, Germany

The tautomeric distributions of trehalulose (**2**), turanose (**3**), maltulose (**4**), leucrose (**5**), and palatinose (**6**) have been established using an ^{1}H NMR methodology based on integration of the low-field anomeric OH singlets in dimethyl sulphoxide. Equilibration in dimethyl sulphoxide being exceedingly slow the method can be applied to determine tautomeric ratios in essentially any solvent, by freezing a small probe of the respective solution (liquid N_2), dissolving the resulting ice-matrix in $(CD_3)_2SO$ and rapid recording of the intensities in the low-field OH-region. In *dimethyl sulphoxide*, fructose, trehalulose (**2**), turanose (**3**), and maltulose (**4**) adopt equilibria with a high proportion of the furanoid tautomers (~ 60% at 20 °C) in an approximate 2:1 β-*f*/α-*f*-ratio versus 10:1 for the β-*p*/α-*p*-forms. For leucrose (**5**), the β-*p* ⇌ α-*p* equilibrium lies at the β-*p* side with a 9:1 preference, whereas palatinose (**6**) establishes an 11:4 β-*f* ⇌ α-*f* equilibrium with a comparably high proportion of the acyclic keto-form (6%), that, in all other cases, is ⩽ 1%. There is little temperature dependence of the anomeric compositions in the 20–70 °C range. In *water*, the β-*p* form invariably is the most abundant at equilibrium, decreasing substantially though with rising temperature in favour of the β-*f* and α-*f*-tautomers. The equilibrium compositions of fructose, trehalulose, and maltulose being almost identical, that of turanose is markedly shifted towards higher proportions of furanoid forms, amounting to two-thirds of the mixture at 70 °C. Aqueous solutions of leucrose contain the β-*p* form almost exclusively, palatinose establishes a 5:1 to 3:1 β-*f* ⇌ α-*f* equilibrium over the 1–70 °C range. In *pyridine*, equilibrium tautomeric compositions are similar to those found for water, except for distinctly slower tautomerization rates. The preparative implications for derivatizations of these glycosyl-fructoses in pyridine are discussed, their now predictable outcome being strongly dependent on the mode of dissolution of the substrate, and the temperature of the reaction.

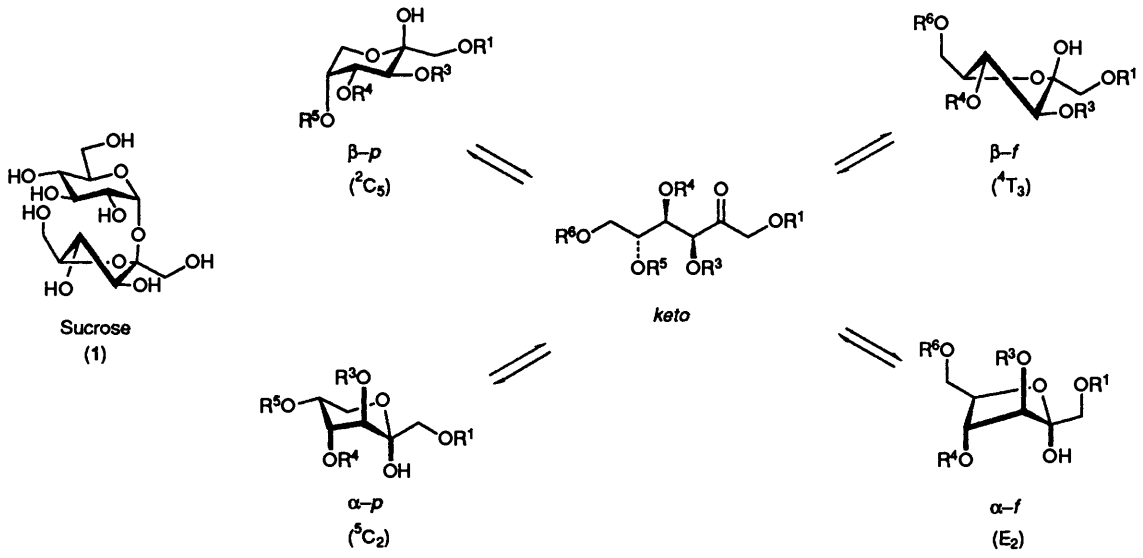
Over the past 30 years, Hough's many excellent contributions to the chemistry of sucrose have vastly expanded our knowledge of this most abundant of natural sugars.[2] In contrast the chemistry of the five sucrose isomers (**2**)–(**6**) is largely undeveloped, the lack of systematic chemical investigations being mainly due to their comparative inaccessibility. Thus, the α(1→1)-linked trehalulose (**2**) has only recently been secured in amounts sufficient for chemical exploitation,[3] the access to turanose (**3**) is reliant on the hydrolysis of manna- and honey-derived melezitose,[4] and maltulose (**4**) may labouriously be acquired from maltose by alkali-induced isomerization.[5–7] Only the α(1→5)-linked leucrose (**5**) and its α(1→6)-analogue palatinose (**6**) have the prospect of becoming available on a very large scale by biotechnological processes, involving the transfer of the D-glucose portion of sucrose from *O*-2 of fructose to either *O*-5 with dextransucrase[8] or to *O*-6 through a bacterial transglucosylases;[9–12] see the Scheme.

Another major impediment against a straightforward exploration of the chemistry of these glucosyl-fructoses lies in the fact, that, on dissolution, complex tautomeric mixtures are formed, the compositions of which vary widely with solvent, temperature, and the time passed after dissolution. Therefore, when subjected to ensuing reactions, random mixtures of products are obtained that are encumbered with little tendency to crystallize and are difficult to separate by chromatography.

One prerequisite for conducting ensuing reactions of (**2**)–(**6**) in a more uniform and predictable way is a detailed knowledge of the distribution of the individual tautomeric forms in a given solvent as a function of time (after dissolution) and temperature—information, that is only sporadically available, and only for water, in the form of ^{13}C NMR-derived equilibrium tautomeric compositions for turanose (**3**) at 36 °C,[16] for maltulose (**4**) at 25, 32, and 60 °C,[17,18] and for palatinose (**6**) at 30 and 65 °C.[19] This situation has led us to examine the tautomeric ratios of all five α-D-glucosyl-D-fructoses (**2**)–(**6**) not only in water, but in organic solvents in which chemistry may be done, *i.e.* in dimethyl sulphoxide, and in pyridine as a function of time and temperature; the results obtained by utilization of the convenient ^{1}H NMR-based methodology developed previously[17–19] are herein presented together with their implications on the acquisition of products in ensuing reactions.

Results and Discussion

Methodology.—For determination of the composition of tautomers, the ^{1}H NMR-based technique used previously for D-fructose[20–22] is used on the premise that the *O*-glucosylated fructoses (**2**)–(**6**) would similarly yield to this method, inasmuch as each has a free anomeric centre. The technique is based on the fact that in dimethyl sulphoxide as the ^{1}H NMR solvent, the anomeric 2-OH protons of furanoid and pyranoid tautomers of D-fructose—and, expectedly, of glucosylated fructoses as well—give distinct singlets in the low-field region of 5.2–6.2 ppm, clearly separated from the doublets and triplets given for secondary and primary OH-resonances, and from the CH-signals as well.[20–22] Indeed, as amply demonstrated by the data in Figure 1, not only D-fructose, but also the five glucosyl-fructoses (**2**)–(**6**) give low-field anomeric OH signals in dimethyl sulphoxide that on integration provide an exact measure of the tautomeric compositon.



Saccharide	R^1	R^3	R^4	R^5	R^6
Fructose	H	H	H	H	H
Trehalulose (2)	Glu	H	H	H	H
Turanose (3)	H	Glu	H	H	H
Maltulose (4)	H	H	Glu	H	H
Leucrose (5)	H	H	H	Glu	–
Palatinose (6)	H	H	H	–	Glu

Scheme. The $^4T_3$ form for the β-*f* tautomer is inferred from the conformation fructose adopts in sucrose (**1**)[13] and β-D-palatinose (**6**),[14] as established by X-ray analysis; the envelope conformation implemented here for the α-*f* isomer is derived from an essentially perfect E_2 form realized in 1,3,4,6-tetra-*O*-benzoyl-α-D-fructofuranose in the crystal (X-ray) and in solution.[15]

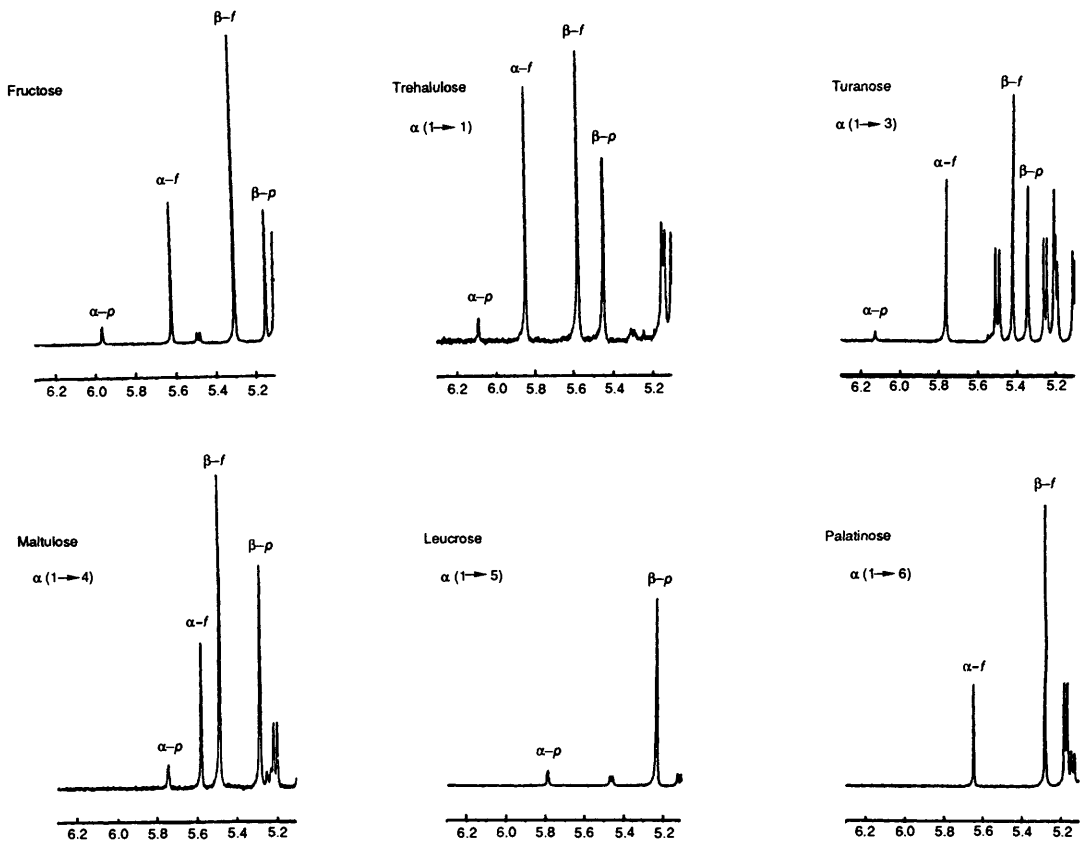


Figure 1. The low-field region of anomeric 2-OH singlets in $(CD_3)_2$ SO at 20 °C after reaching equilibrium (6 d).

Table 1. Equilibrium composition of tautomers in dimethyl sulphoxide for D-fructose and the α-D-glucopyranosyl-D-fructoses (**2**)–(**6**).

Saccharide	T/°C	Time[a]	Tautomers (%) α-p	α-f	β-f	β-p	keto	Ref.
Fructose	20	7 d	3.4	19.1	45.5	32.0	—	
	20	?	5	20	46	29	—	21
	20	?	3.6	19.3	45.6	31.5	—	24[b]
	20	24 h	5	19	45	31	—	25[c]
	25	53 d	4.8	20.1	48.1	27.0	—	22
	30	5 d	5.0	21.1	48.6	25.3	—	
	30	?	5	21	48	26	—	21
	40	4 d	4.4	22.8	49.3	23.5	—	
	40	?	4.5	22.5	50	23	—	21
	50	12 h	4.5	23.0	51.0	21.5	—	
	50	?	4.5	23.5	51	21	—	21
Trehalulose (**2**)	20	2 d	3.5	24.0	38.2	34.3	—	
	40	4 d	3.5	24.0	38.8	33.7	—	
	70	3 d	3.5	24.7	41.8	30.0	—	
Turanose (**3**)	20	4 d	2.8	23.4	36.9	36.9	—	
	40	4 d	2.6	24.3	39.1	34.0	—	
	70	3 d	2.8	26.7	42.2	28.3	—	
Maltulose (**4**)	20	6 d	4.9	17.6	42.4	35.1	—	
	25	2 d	4.2	21.6	39.6	34.6	—	19
	40	4 d	3.5	18.3	40.9	37.3	—	
	70	3 d	2.7	20.4	43.4	33.5	—	
Leucrose (**5**)	20	12 d	8.9	—	—	89.3	1.8[d]	
	40	4 d	10.4	—	—	89.6	<1	
	55	3 d	10.8	—	—	89.2	<1	
Palatinose (**6**)	20	3 d	—	25.0	69.1	—	5.9[d]	
	40	4 d	—	24.7	71.6	—	3.7	
	55	3 d	—	24.8	72.0	—	3.2	

[a] Time past between dissolution and ^{1}H NMR recording; the rate at which the equilibrium is established clearly depends on solvent purity; traces of water in the $(CD_3)_2SO$ used substantially enhance equilibration. [b] In ref. 24 (Table 2, footnote b) the OH intensity-derived equilibrium values given are 19.3% for the β-*p* and 31.5% for the α-*f* tautomer; if interchanged, as here, the values correlate well with ours and those of Nicole *et al.*[21] [c] ^{13}C NMR-based data determined at 'ambient probe-temperature'[25] which must have been close to 20 °C in view of their close correlation with the ^{1}H NMR derived values of ours and Nicole.[21] [d] Values derived from the signal intensity of the 3-OH doublet of the acyclic keto-form, appearing well separated at comparably high field [4.73 ppm for (**5**) and 4.58 for (**6**)].

Unequivocal correlation of these low-field OH singlets with the anomeric OH groups of the respective tautomeric form is based on the following pieces of evidence: (*i*) crystalline β-D-fructopyranose, 3 min after dissolution in dimethyl sulphoxide, only shows the OH resonance at δ 5.14 (*cf.* Figure 1) indicating that the β-*p*-OH resonance appears at higher field than any of the others; (*ii*) palatinose (**6**), as a 6-*O*-glucosylated fructose only capable of adopting furanoid forms, similarly gives only one OH singlet (at 5.26) when running the ^{1}H NMR spectrum minutes after dissolution in dimethyl sulphoxide; this signal must originate from the β-*f* OH resonance, since (**6**) crystallizes in the β-*f* form as evidenced by X-ray analysis;[14] if (**6**) is left in solutions another OH singlet at lower field (δ 5.64) gradually develops, obviously the α-*f*-OH resonance due to increasing β-*f* $\rightleftharpoons$ α-*f* tautomerization, equilibrium being reached after 6 d with the β-*f* anomer still strongly preponderating (*cf.* Figure 1); (*iii*) leucrose (**5**), in turn, which due to 5-*O*-glucosylation of the fructose can only establish a β-*p* $\rightleftharpoons$ α-*p* equilibration, exclusively shows the high-field β-*p*-OH singlet directly after solution—in accord with X-ray structural data[23] that prove the β-*p* form for (**5**) in the crystalline state; only after being left to stand for days in demethyl sulphoxide solution does the lower-field α-*p*-OH derived resonance gradually appear (Figure 1). These assignments, in themselves reciprocally conclusive, are further substantiated by the fact, that superposition of the anomeric OH regions of leucrose (**5**) and palatinose (**6**) results in four consecutive OH singlets with chemical shifts very close to those obtained for the same signals in trehalulose (**2**), turanose (**3**), and maltulose (**4**). In addition, the equilibrium composition of maltulose tautomers (Figure 1 and Table 1) closely resembles that of a conceivable mixture of (**5**) and (**6**) arrived at by superimposing the respective anomeric OH signals.

The ^{1}H NMR-based methodology used here combines modest technical expenditure, short measuring time with simple application to any temperature desired, yet cannot take hold of the acyclic keto form, present in solution. It may be derived though—at higher expenditure—from the peak area of its ^{13}C-2 carbonyl resonance, and is in the range of 1% for dimethyl sulphoxide. In the case of leucrose (**5**) and palatinose (**6**), however, due to their adoption of pyranoid and furanoid forms only, distinct OH-derived doublets appear at 4.73 (**5**) and 4.58 ppm (**6**), of low intensity and not assignable to secondary ring-OH groups, that in view of their appearance at comparably high field are most likely to result from the 3-OH of the acyclic keto-form. Accordingly, the tautomeric equilibrium composition for (**5**) and (**6**), was calculated from the peak intensities of the two anomeric OH-singlets and these doublets (*cf.* Table 1 and Figure 2), indicating a 1.8% keto-form for (**5**) at 20 °C, decreasing at higher temperature, whilst palatinose appears to have more of the acyclic tautomer present in the mixture (3–6%) than any of the other disaccharides or fructose itself. The percentage values at 20 °C have been corroborated by ^{13}C NMR data which are somewhat lower though [1% for (**5**), and 2.6% for (**6**)] obviously due to a higher error involved in determining the peak area of the C-2 resonance.

Tautomeric Distribution of α-D-*Glucosyl-fructoses* (**2**)–(**6**) *in Dimethyl Sulphoxide.*—The correlation between low-field anomeric OH-signals and tautomeric form thus being reliably secured, the methodology was applied to the determination of tautomeric ratios as a function of time and of temperature. Representative for all of the glucosylfructoses is the time course observed for maltulose (**4**), detailed in Figure 2. Starting from the crystalline β-*p* tautomer, only this form is present right after dissolution in dimethyl sulphoxide. It decreases gradually on expense of the other three tautomers, the β-*p* proportion falling below 50% after 30 h and to *ca.* 40% after several days, the end point at 20 °C being reached after *ca.* one week.

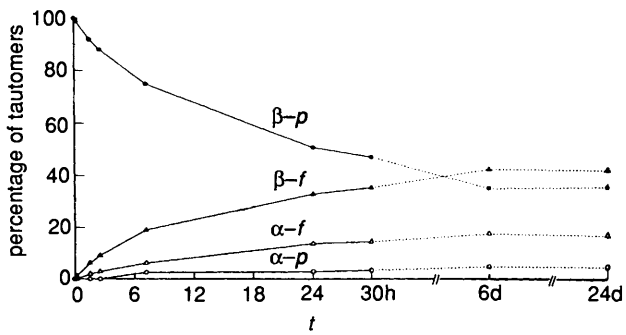


Figure 2. Composition of D-maltulose tautomers in dimethyl sulphoxide at 20 °C as a function of time, starting from the crystalline β-*p* form.

Similarly slow tautomerization rate profiles are obtained when starting from crystalline β-*p*-leucrose and β-*f*-palatinose, respectively. In the case of trehalulose (**2**), the time course of equilibration could not concisely be determined because the disaccharide has only been obtained in syrupy form (from

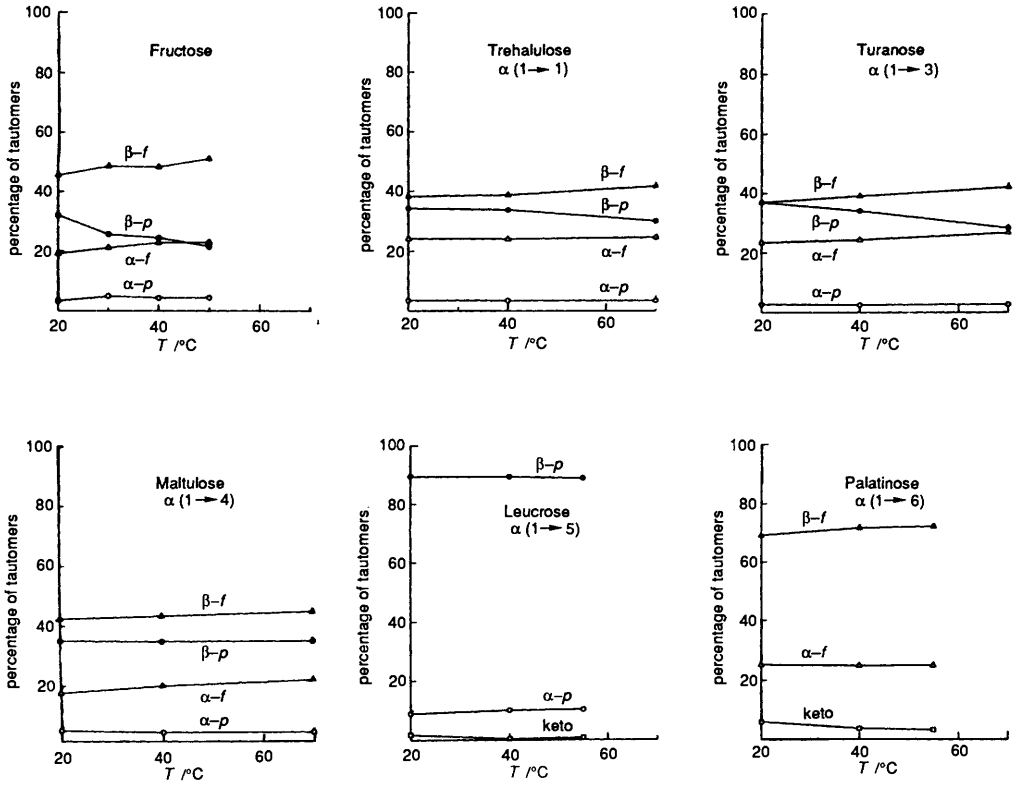


Figure 3. Equilibrium composition of tautomers in $(CD_3)_2SO$ of D-fructose, trehalulose (**2**), turanose (**3**), maltulose (**4**), leucrose (**5**), and palatinose (**6**) as a function of temperature.

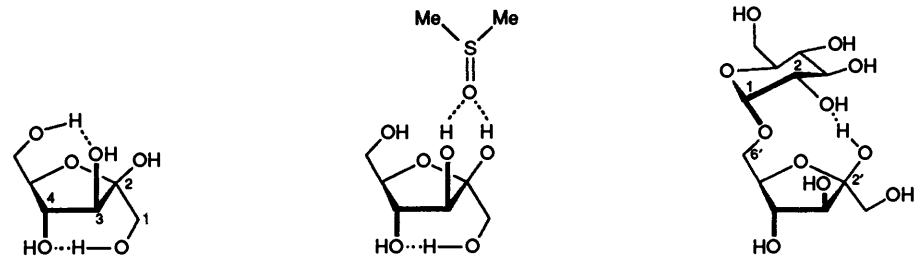
water), and, as such, constitutes a mixture of all four tautomers.

Equilibration of tautomers in dimethyl sulphoxide being exceedingly slow, this simple and reliable method for the rapid determination of tautomeric ratios can be applied to any temperature—provided the disaccharide is stable thereby—thus allowing the determination of the temperature dependence of anomeric ratios by simply cooling the probe to ambient temperature and rapid recording of the OH signals and subsequent integration. The results thus obtained are collected in Table 1, spanning the 20–70 °C range only; lower temperature is prohibitive due to solidification of dimethyl sulphoxide at 18.5 °C, and higher temperatures are inopportune to noticeable (^{1}H NMR) decomposition particularly in the case of D-fructose and palatinose, increasingly forming furane derivatives.

As amply demonstrated by the data of Table 1 and their graphical presentation in Figure 3, the temperature effect on tautomeric ratios, throughout, is not very pronounced. In the case of D-fructose, the β-*f*-tautomer, already prevalent in the equilibrium mixture at 20 °C, increases from 45–51% on raising the temperature to 50 °C, as does the α-*f*-form (19 to 23%), at the expense of β-D-fructopyranose (32 to 21%). Thus, at 50 °C, dimethyl sulphoxide solutions of D-fructose contain 75% of the furanoid tautomers, nourishing expectations that the α-*f* or β-*f* form may conceivably be induced to crystallize on cooling or, after freeze-drying, by trituration of the residue with solvents in which tautomerization is slow; however, attempts along this line have not materialized so far.

The temperature dependence in the distribution of D-fructose tautomers is similarly found for turanose (**3**): β-*f* + 5%, α-*f* + 4%, β-*p* −9% on going from 20 to 70 °C, and to a lesser degree for trehalulose (**2**) and maltulose (**4**) indicating no major differences brought about by glycosylation of fructose at O-1′, O-3′ or O-4′. Leucrose (**5**), in contrast, exhibits no temperature dependence at all for the β-*p* $\rightleftharpoons$ α-*p* equilibrium between 20 and 70 °C, while in palatinose (**6**), the β-*f* form slightly increases with higher temperature, on expense of the acyclic tautomer (*cf.* Table 1 and Figure 3).

To account for the high proportion of the β-furanose form of D-fructose in dimethyl sulphoxide (as compared with that in water, see below), an intramolecular association between pairs of primary and secondary hydroxy groups has been proposed,[24] *i.e.* hydrogen-bonding between 1-OH and 4-OH, and 6-OH and 3-OH, respectively [formula (**A**) of Figure 4]. If so, blocking of any of these hydrogen-bonded hydroxy groups, *e.g.* by glucosylation, should result in a decrease of the β-*f* proportion relative to the other tautomeric forms. Inspection of the 20 °C data in Table 1 indeed shows a lesser population of the β-*f* form for trehalulose (−8% relative to fructose), turanose (−9%) and maltulose (−4%). Whether this effect, however, is due to perturbation of the intramolecular hydrogen bonds as in (**A**) or is to be attributed to the presence of intersaccharidic hydrogen bonds, of which many are conceivable on the basis of molecular models, remains an open question. Palatinose (**6**), for example, establishes a hydrogen bond between the anomeric fructosyl-OH and the 2-OH of the glucose portion in the crystalline state[14] [Figure 4, (**C**)], which may survive solution and equilibration in dimethyl sulphoxide to account for the approximate 3:1 preference of the β-*f* form (69% at 20 °C, *cf.* Table 1) over the α-*f*-tautomer. Clearly, a considerably deeper understanding of the solvatization processes of polyhydroxylated systems in dimethyl sulphoxide is required, *i.e.* whether complexes are formed as discussed for some sugars,[26] or to what extent the oxygen of dimethyl sulphoxide forms hydrogen bonds with one hydroxyl proton or, as proposed by Casu *et al.*,[27] with the hydrogens of two vicinally *cis*-disposed hydroxyl groups [Figure 4, (**B**)]. Glucosylated fructoses, at least, provide no clear answer as to the nature of solvatization in dimethyl sulphoxide.



(A) (B) (C)

Figure 4. (**A**) The β-furanose form of D-fructose stabilized by two intramolecular hydrogen bonds between primary and secondary hydroxy groups.[20] (**B**) Conceivable dimethyl sulphoxide ••• *cis*-diol hydrogen bonding in the β-*f*-tautomer of fructose.[27] (**C**) Palatinose (**6**) in the crystalline state,[22] depicting the intersaccharide hydrogen bond between the anomeric OH of fructose and the 2-hydroxy of the glucose moiety.

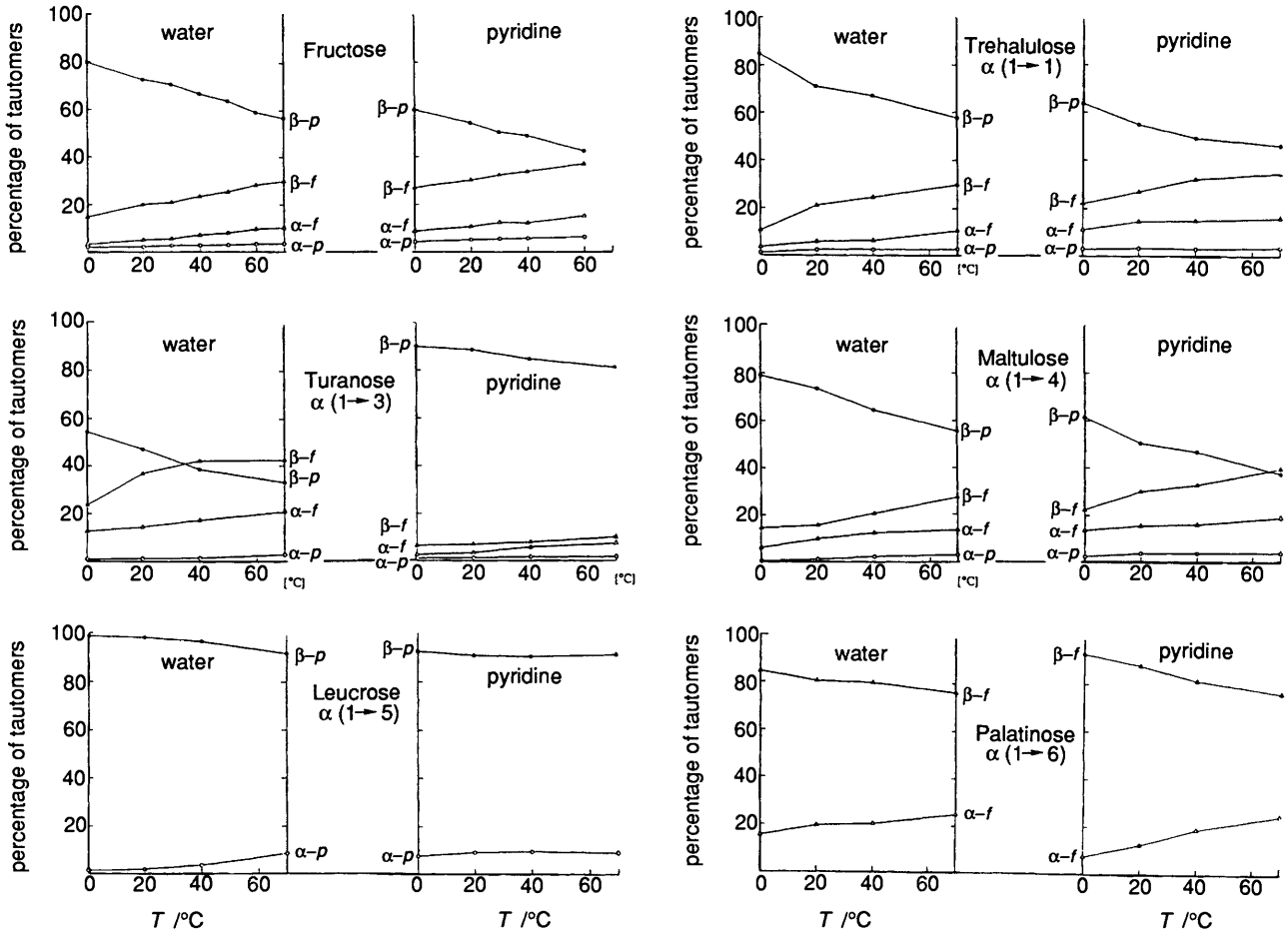


Figure 5. Equilibrium composition of tautomers of D-fructose and the five α-D-glucopyranosyl-D-fructoses (**2**)–(**6**) in water (left) and pyridine (right) as a function of temperature.

α-D-*Glucosyl-fructoses* (**2**)–(**6**) *in Water*.—Due to the very slow tautomerization of D-fructose and its glucosyl derivatives (**2**)–(**6**) in dimethyl sulphoxide—within the 2 min required for recording the ^{1}H NMR spectrum, changes at 20 °C are essentially non-detectable—the 'anomeric OH intensity' methodology can be extended to other solvents, simply by taking a small sample (0.07 cm^3) of the respective glucosyl-fructose solution in a given solvent, interrupting further isomerization through freezing (liquid N_2), dissolving the ice-matrix in $(CD_3)_2SO$ and rapidly recording the ^{1}H NMR signals in the low-field OH region. One prerequisite for the success of the method naturally is that the small proportions of other solvents introduced into the dimethyl sulphoxide solution do not adversely affect tautomeric distributions. In order to eliminate possible influences exerted by the new solvent system (dimethyl sulphoxide with up to 6% of water or pyridine), and to account for the time lag between dissolution of the frozen sample in dimethyl sulphoxide (t_0) and ^{1}H NMR recording (t_1), a second run was carried out after 15 min (t_2), and both data sets were then used for extrapolation towards t_0. The intensity changes between t_1 and t_2, however, never exceeded 2%.

The data obtained in this way for aqueous solutions of D-fructose[22] and the five glucosyl-fructoses (**2**)–(**6**) in the 1–70 °C range at equilibrium are presented in Figure 5 and Table 2.

The rate of tautomerization is—unlike dimethyl sulphoxide—very fast. In the case of D-fructose, equilibrium at ambient

Table 2. Equilibrium composition in water and pyridine for D-fructose and its *O*-glucosylated derivatives (2)–(6).

Saccharide	Solvent	T/°C	Time[a]	Tautomers[b] (%) α-*p*	α-*f*	β-*f*	β-*p*	Ref.
Fructose	water[c]	1	3 h	2.1	3.3	14.8	79.8	22
		25	2 h	2.3	5.2	20.0	72.5	22
		30	1 h	2.9	5.7	20.9	70.5	22
		30[d]	?	2	5	23	70	28
		31[d]	?	2.6	6.5	25.3	64.8	29
		40	30 min	2.8	7.2	23.4	66.6	22
		50	20 min	3.0	8.1	25.3	63.6	22
		60	15 min	3.4	9.7	28.1	58.8	22
		70	7 min	3.6	10.3	29.7	56.4	22
	pyridine	1	7 d	4.4	8.9	26.9	59.9	22
		20	2 d	5.2	10.6	30.0	54.1	22
		30		5.5	12.3	32.0	50.1	22
		40	3 h	5.7	12.2	33.5	48.6	22
		60	1 h	6.2	15.0	36.7	42.1	22
Trehalulose (2)	water	1	3 h	1.1	3.5	10.5	84.9	
		20	1 h	2.3	5.7	21.1	70.9	
		40	15 min	2.4	6.2	24.5	66.9	
		70	7 min	2.6	10.4	29.6	57.4	
	pyridine	1	12 h	3.0	11.2	22.1	63.7	
		20	3 h	3.4	14.7	27.0	54.9	
		40	1 h	3.3	15.1	32.3	49.3	
		70	30 min	3.5	16.0	34.5	46.0	
Turanose (3)	water	1	1 h	1.0	12.7	32.7	53.6	
		20	30 min	1.4	14.5	36.8	47.3	
		36		<4	20	41	39	16
		40	20 min	1.6	17.4	42.3	38.7	
		70	7 min	3.1	21.0	42.6	33.3	
	pyridine	1	18 h	1.1	2.6	6.5	89.8	
		20	15 h	1.3	3.4	7.0	88.3	
		40	1 h	1.8	5.9	8.0	84.5	
		70	20 min	1.8	7.4	10.0	80.8	
Maltulose (4)	water	1	2 h	0.5	6.1	14.5	78.9	
		20	1 h	1.2	9.8	15.6	73.4	
		25[d]		1.5	12.1	22.4	64.0	18
		32		—	7.6	30.7	61.5	17
		40	40 min	2.4	12.5	20.7	64.4	
		58[d]		2.1	14.3	29.6	55.0	18
		60		—	14.3	42.9	42.9	17
		70	7 min	3.1	13.7	27.7	55.5	
	pyridine	1	7 h	2.4	13.7	22.4	61.5	
		20	4 h	3.9	15.7	30.1	50.3	
		40	40 min	4.0	16.3	32.9	46.7	
		70	30 min	4.0	19.1	39.5	37.4	
Leucrose (5)	water	1	12 h	1.3			98.7	
		20	40 min	1.9			98.1	
		40	25 min	3.5			96.5	
		70	5 min	8.6			91.4	
	pyridine	1	5 h	7.6			92.4	
		20	4 h	9.2			90.8	
		40	1 h	9.7			90.3	
		70	7 min	9.0			91.0	
Palatinose (6)	water	1	12 h	—	15.5	84.5		
		20	30 min	—	19.7	80.3		
		30	?	—	28.6	71.4		17
		40	20 min	—	20.5	79.5		
		65	?	—	25.0	75.0		17
		70	7 min	—	24.4	75.6		
	pyridine	1	12 h	—	7.6	92.4		
		20	3 h	—	12.5	87.5		
		40	1 h	—	18.8	81.2		
		70	30 min	—	24.3	75.7		

[a] Time past between dissolution and interruption of tautomerization by freezing. [b] The (acyclic) keto form, not detectable by the ^{1}H NMR-based methodology used here, has been determine *via* its ^{13}C-2 carbonyl resonance to be in the 0.5–2% range for D-fructose,[29–31] and 1.5–2.1 ± 0.5% for maltulose.[18] [c] For D-fructose in D_2O there are other ^{13}C NMR determinations of the tautomeric composition at equilibrium based either on peak areas of the anomeric carbon atoms only, or on areas of all well separated carbon atoms, or both, using between 5 000 to 100 000 scans;[31–33] the data thus obtained correlate less well with ours, as do those derived from GLC.[34] [d] Data derived from ^{13}C NMR measurements.

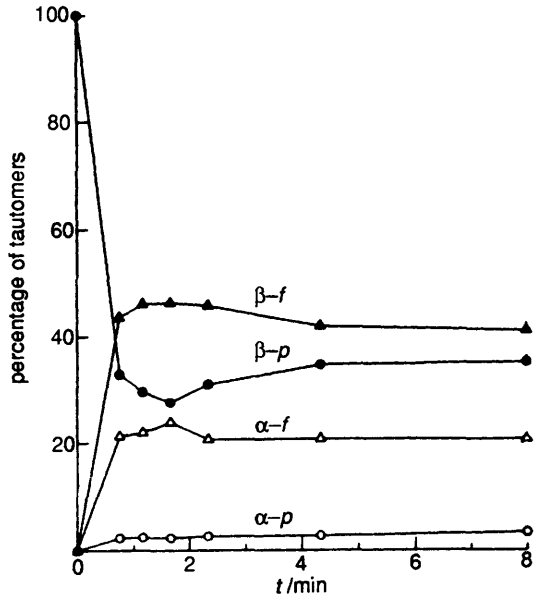
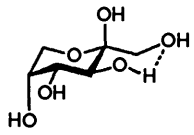


Figure 6. Percent composition of turanose tautomers in water at 70 °C as a function of time, starting by dissolution of the crystalline β-*p* form.

temperature is reached within 1–2 h,[35] which also holds for any of the glucosylated fructoses (**2**)–(**6**). Equilibration becomes faster on raising the temperature; at 70 °C, for example, a constant composition of tautomers is observed after a few minutes. For turanose (**3**), an abnormal time course was found for its tautomerization in water at any of the temperatures measured, becoming most pronounced at 70 °C (*cf.* Figure 6): the decline of the β-*p*-form with which the experiment was started, reaches a lower value (28% after 2 min) than that at equilibrium (33% after 4 min), an effect that is similarly borne out by corresponding maxima in the rate profiles for the β-*f* and α-*f* tautomers. When comparing the equilibrium composition of D-fructose with that of the five *O*-glucosyl-fructoses (**2**)–(**6**) (Figure 5 and Table 2), it becomes apparent that D-fructose, trehalulose (**2**) and maltulose (**4**) show a close resemblance in their tautomeric distributions over the entire 1–70 °C range, differences being within a few percent only. Accordingly, it can be concluded, that substitution at O^1 and O^4 of fructose has very little effect on the comparative stabilities of pyranoid and furanoid tautomeric forms.

Distinctly different is turanose (**3**); the proportion of the β-*p* form relative to D-fructose, is substantially smaller (47% at 20 °C *versus* 71% for D-fructose), the β-*f*-tautomer becoming the prevailing one on going to higher temperature (43% β-*f versus* 33% β-*p* at 70 °C, *cf.* Table 2). A similar destabilization of the β-*p*-tautomer has been observed for 3-*O*-methyl-D-fructose, for which ^{13}C NMR based data[36] reveal nearly equal proportions of β-*p*- and β-*f*-forms in aqueous solution (37 and 34%,* respectively at 16.5 °C). Thus, O^3-substitution of D-fructose results in a substantial pertubation of the equilibrium tautomeric composition in water (and in pyridine as well, *cf.* below), such, that the β-*p* form is destabilized in favour of both of the furanoid tautomers. An enticing rationalization for the high β-*p* proportion in D-fructose (as well as in maltulose or leucrose) would be the presence of an intramolecular hydrogen bond between the primary 1-OH and the 3-OH, as indicated in the formula—a stabilizing factor for the β-fructopyranose form



that is lost on 3-*O*-methylation or 3-*O*-glucosylation. However, trehalulose (**2**), in aqueous solution, exhibits a fructose-like distribution pattern of tautomers, clearly indicating that blocking of the 1-OH in fructose has no complementary effect.

In water, the β-*p* ⇌ α-*p* equilibrium for leucrose (**5**) strongly lies on the β-*p* side, sizable proportions of the α-*p* tautomer only being present at higher temperatures (9% at 70%C). The corresponding β-*f* ⇌ α-*f* equilibration of palatinose (**6**), similarly shows the β-*f*-tautomer to preponderate with 4:1 ratio in the 20–40 °C range (Figure 5), a ratio that is changed insignificantly on increasing the temperature.

Glucosyl-fructoses (**2**)–(**6**) *in Pyridine.*—Many derivatizations of fructose and, conceivably, of its glucosylated derivatives being performed in pyridine as the solvent information on the rate of isomerization as a function of temperature and on the tautomeric compositions at different temperatures appeared to be particularly important. For determination of the equilibrium composition of tautomers, saturated solutions of the β-fructopyranose in pyridine were kept at the respective temperature until equilibrium was reached, subsequently frozen, transferred into $(CD_3)_2SO$ solution and the NMR spectra recorded. The resulting characteristic singlets for the anomeric hydroxy groups are slightly shifted towards lower field as compared with the water–dimethyl sulphoxide system but are readily integrated.

The equilibration rates were secured only insofar as the times listed in Table 2 between dissolution in pyridine and interruption of tautomerization by freezing were truly those at equilibrium, later recordings of anomeric OH intensities showing no change in the percentage composition of the individual tautomers. The rate of formation of the other isomeric form from the β-*p*-tautomer, with which equilibration is started in the case of fructose, turanose (**3**), maltulose (**4**) and leucrose (**5**), is strongly temperature-dependant: 70 °C equilibrium is reached within 20–30 min, at 1 °C, respectively, it is a matter of 12–24 h, provided the pyridine used is absolutely dry; in general, the rate of tautomerization in pyridine is distinctly slower than in water, yet decisively faster than in dimethyl sulphoxide. The rate profiles are linear, except for turanose (**3**) which gives a time course for its pyridine equilibration analogous to that observed for water (*cf.* Figure 6), *i.e.* the decline of the β-*p* form proceed through a minimum with a complementary course of the two furanoid tautomers generated thereby. Obviously, the rates with which the four equilibria cyclic tautomer ⇌ acyclic keto-form are adjusted are substantially different for turanose (**3**) than for any of the other disaccharides (**2**) and (**4**)–(**6**) and fructose.

The equilibrium tautomeric compositions obtained in this way for fructose and its glucosylated analogues (**2**)–(**6**) are listed in Table 2 and are graphically presented in Figure 5. Throughout, the β-*p*-form is the predominant tautomer over the entire 1–70 °C range decreasing on rising temperature in favour of the β-*f*- and α-*f*-forms, with little effect on the proportion of the α-*p*-tautomer. It is interesting to note, that the equilibrium tautomeric composition of fructose, of trehalulose (**2**) and of maltulose (**4**) are essentially the same over the entire 1–70 °C temperature range, in the case of the latter (**4**), the β-*f*- and β-*p*-forms reaching comparable stability at 70 °C, then being present to *ca.* 40% each. Leucrose (**5**) shows no temperature dependence for its β-*p* ⇌ α-*p* equilibrium set at a 9:1 preference for the β-*p*-form. Palatinose (**6**), in turn, exhibits a β-*f*:α-*f* ratio of 9:1 at low temperature that is diminishing to 3:1 at 70 °C.

* The proportions found for the respective α-anomers (18% α-*p*, 11% α-*f*),[36] however, differ markedly from those found for turanose (1.4% α-*p* and 14.5% α-*f* at 20 °C) which appears difficult to rationalize.

When comparing the data obtained for pyridine with those in water (*cf.* Figure 5) the respective tautomeric distributions as well as their temperature dependence are surprisingly analogous, clearly pointing towards very similar modes of solvation in the two solvents. A notable exception is turanose (**3**):* in pyridine, the β-*p*-form is distinctly favoured (88% at 20 °C, 81% at 70 °C), in water the β-*p*-form is populated to only about half (47% at 20 °C, 33% at 70 °C), on expense of the β-*f*-tautomer, which becomes the major tautomer from 35 °C on (Figure 5). A juxtaposition of the combined proportions of the two furanoid forms *versus* their pyranoid tautomers—in water at 20 °C, 51% of the furanoid forms are present in the equilibrium mixture, in pyridine only 10%—also demonstrates the anomalous solvatization behaviour of turanose in the two solvents relative to fructose and its glycosyl-analogue (**2**), and (**4**)–(**6**).

Preparative Implications.—The knowledge elaborated here on the behaviour of D-fructose and its five α-D-glucosyl derivatives (**2**)–(**6**) is of considerable preparative importance for planning derivatizations aimed at preparative yields of furanoid or pyranoid products. Acylations, for example, with pyridine–acetic anhydride or benzoyl chloride in pyridine will give satisfactory yields of β-pyranoid products only, when a maximum of the respective β-*p*-form is present *a priori*; on the basis of the results in Table 2 and Figure 5, this not only means low reaction temperature (*i.e.* 0 °C), but, in addition, dissolving of the starting material in pyridine at 0 °C, which usually requires hours. Otherwise, on dissolving a crystalline β-*p*-glucosyl-fructose, in pyridine at ambient temperature, for example, or, even worse, when speeding up the dissolving process by warming followed by cooling and addition of the reagents the product distribution will be distinctly more complex, its exact composition depending on the time the starting material stood in pyridine before starting the reaction.

The most favourable conditions for the acquisition of pyranoid products from fructose as well as the glucosylfructoses (**2**)–(**5**), hence, reacts in pyridine at low temperature (0 °C), after patiently dissolving the substrate in this solvent at 0 °C. In turn, furanoid derivatives may advantageously be obtained by using pyridine or dimethyl sulphoxide at 50–70 °C. This rationalization is supported by the experimental finding that 1,3,4,6-tetra-*O*-benzoyl-α-D-fructofuranose is obtained in surprisingly high yield (59%) on exposure of fructose to pyridine–benzoyl chloride at 60–65 °C.[37] At this temperature, the 'fructose' in pyridine consists of approximately half of the α-*f*- and β-*f*-forms (*cf.* Figure 5), in which the primary hydroxy groups are obviously benzoylated first—thereby irreversibly fixing the products in the furanoid form—followed by re-establishment of the equilibrium. The application of these conditions to trehalulose, turanose, maltulose, and leucrose for the high-yield generation of furanoid products is obvious—experiments on which we hope to report in due course.

Experimental

Origin of Glucosyl-fructoses.—Trehalulose (**2**) and palatinose (**6**) were prepared from sucrose by *Protaminobacter rubrum*-mediated transglucosylation, *i.e.* transfer of the glucose portion from its *O*-2′-fructose linkage to *O*-1′ (→2)[2] and *O*-6′ (→6),[11] respectively, and were obtained from Südzucker. Turanose (**3**) was used in its commercially available form (Fluka), the leucrose (**5**) employed was a product prepared by dextransucrase-induced glycosyl-transfer from sucrose to *O*-5′ of fructose[8] and provided by Pfeifer & Langen. Maltulose (**4**) was most efficiently prepared by sodium aluminate-mediated isomerization of maltose in a procedure, adapted from a patent;[7] it involves heating of an aqueous solution of maltose, $NaAlO_2$ (0.35 mol each in 400 cm^3) and sodium hydroxide (5 g) for 4 h to 45 °C, and processing of the mixture by acidification with 5 mol dm^{-3} sulphuric acid (to pH 3.8), addition of $CaCO_3$ until formation of CO_2 ceases (to pH 6.7), filtration, and deionization (*e.g.* Dowex 50, H^+-form and Amberlite IRA 904, OH^--form) followed by concentration and direct crystallization. This methodology appears more practical than the isomerization of maltose with dilute sodium hydroxide[5] or with triethylamine in the presence of boric acid,[6] since it effects a 90% conversion—only 5% of maltose, 2% each of glucose and fructose, and traces of higher saccharides are present in the reaction mixtures on the basis of HPLC—and, upon deionization allows direct crystallization of the maltulose monohydrate from a concentrated aqueous solution in yields of 65–70%.

^1H *NMR Methodology.*—The ^1H NMR Spectra were measured on a Bruker WM 300 instrument. Determination of tautomeric distribution in water and pyridine was effected as follows: a 0.6–0.8 mol dm^3 solution of the individual glucosyl-fructose in water† or pyridine† is kept at constant temperature (1, 20, 40, and 70 °C, respectively) for the time given in Tables 1 and 2, followed by extraction of a 0.07 cm^3 sample that is transferred to an NMR tube and subsequently frozen (liquid N_2), the ice-matrix, thus obtained, is dissolved in $(CD_3)_2SO$ (1 cm^3) and the anomeric OH signals in the 5.2–6.0 ppm region (*cf.* Figure 1) are recorded twice, *i.e.* 3 and 15 min after dissolution (temperature 20 °C, recording time: <2 min). Thereby, the chemical shifts of the respective OH singlets in dimethyl sulphoxide (*cf.* Figure 1) are shifted slightly to higher field (by 0.1–0.2 ppm) in the DMSO –6% pyridine and DMSO–6% water cases. For the two measurements each, the tautomeric distribution is determined by integration of the respective low-field anomeric OH singlets and by linear extrapolation of the 3 and 15 min values to the time of dissolution of the frozen sample in dimethyl sulphoxide. The differences in intensity between the 3 and 15 min values were usually in the 1% range and never exceeded 2%.

The data thus obtained are listed in Tables 1 and 2, and are graphically represented in Figures 2 and 5.

† The use of deuteriated water or C_5D_5N is unnecessary as the peak for water in $(CD_3)_2SO$ is at 3.3 ppm, that for the aromatic protons of pyridine at 7.6–8.1 ppm both not obscuring the anomeric OH-region.

* *Note added in proof.*—We have observed similar anomalous solvation behaviour with the readily accessible[37] 3-*O*-methyl-D-fructose. Although it crystallizes in the β-*p* form (like fructose), the equilibrium composition of tautomers in water between 0–70 °C is nearly identical with that of its 3-*O*-glucosyl analogue, turanose (**3**) (*cf.* Figure 5). In pyridine, however, the ratio of tautomers found—8% α-*p*, 25% α-*f*, 41% β-*f*, and 26% β-*p* form at 20 °C with nearly no temperature dependence between 0 and 70 °C, respectively—is completely different from that of fructose, of turanose (**3**), in particular, and of any of the other glucosyl-fructoses. In dimethyl sulphoxide at 20 °C, 77% of the 3-*O*-methyl-fructose adopts the furanoid form (47% β-*f*, 30% α-*f*) with only 3% α-*p* and 20% of the β-*p* tautomer present—the lowest content of pyranoid forms observed for any of the fructoses studied.

Acknowledgements

Professor Leslie Hough is an esteemed scientist, colleague, and friend. We respectfully acknowledge the past research on the chemistry of sucrose carried out by him, to which the work described in this paper is complementary.

The authors are grateful to the *Bundesministerium für Forschung und Technik* for financial support (Grant 0319 251

A); they are also indebted to Dr. H. Schiweck, Südzucker Research Laboratories, Obrigheim, for the provision of trehalulose and palatinose. Thanks are also due to Dr. K. B. Hicks, US Department of Agriculture, Philadelphia, for a sample of authentic maltulose, and to Dr. D. Schwengers, Pfeifer & Langen, Dormagen, for the leucrose.

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Paper 0/00768D
Received 19*th February* 1990
Accepted 3*rd April* 1990