CHARACTERIZATION OF KETO DISACCHARIDES IN SOLUTION BY DEUTERIUM-INDUCED, DIFFERENTIAL ISOTOPE-SHIFT ^{13}C-N.M.R. SPECTROSCOPY*

PHILIP E. PFEFFER AND KEVIN B. HICKS

Eastern Regional Research Center†, Philadelphia, Pennsylvania 19118 (U.S.A.)

ABSTRACT

The chemical-shift assignments and tautomeric distribution of three fructose-containing disaccharides, lactulose, cellobiulose, and maltulose have been established with the ^{13}C deuterium-induced, differential isotope-shift (d.i.s.) technique. A number of ^{13}C resonance assignments, which could not have been made previously, are described for the spectrum of maltulose based on observed isotope perturbations. The observed d.i.s. values for the glycosylated C-4' carbon resonances of the pyranose tautomers were found to be sensitive to the stereochemistry of the glycosidic linkage. Fructopyranose forms dominate the tautomeric distribution of each keto disaccharide, with the percentage of fructofuranose forms increasing with temperature. As much as 2.5% open-chain "keto" form of disaccharide was detected in solution.

INTRODUCTION

Although ^{13}C-n.m.r. spectroscopy in an effective tool for characterizing the tautomeric composition of oligosaccharides, the complete interpretation and assignment of these spectra is difficult to achieve[1]. Often the difficulty in interpretation lies in assignments of the resonances representing the following groups of carbon atoms in specific domains of the spectra; C-2, C-3, and C-5 (ring) of aldopyranoses, C-3, C-4 (ring) of ketofuranoses, and C-1 and C-6 of ketofuranoses. Within each of these three groups, the problem of line differentiation results from the indistinguishability of each resonance in its proton-coupled state, namely, the multiplicity of each proton-coupled resonance within each of these groups is the same. To distinguish these lines from one another, it is necessary either to perform a single frequency, off-resonance decoupling (s.f.o.r.d.) experiment from an unambiguously assigned proton spectrum or resort to isotope-labeling methods. The deuterium-induced, differential isotope-shift (d.i.s.) ^{13}C-n.m.r. technique[1] offers in many instances an alternative, facile

approach to the identification of carbohydrate spectral absorptions without the need to rely on s.f.o.r.d.[2] or complex syntheses with isotopes[3].

Previously, Angyal and Bethel[4], using isotope labeling, we[1], using the d.i.s. method, and Jones et al.[2], using s.f.o.r.d., established the complete assignments for the ^{13}C spectrum of the keto disaccharide sucrose. However, only recently have the ^{13}C spectra of keto oligosaccharides containing reducing-end D-fructose residues been reported[5]. In this study, we present the d.i.s. [13]C assignments and information concerning the solution chemistry of the D-fructose reducing-end disaccharides, lactulose (4-O-β-D-galactopyranosyl-D-fructose), cellobiulose (4-O-β-D-glucopyranosyl-D-fructose), as well as some alternative assignments for the previously described spectrum[5] of maltulose (4-O-α-D-glucopyranosyl-D-fructose).

EXPERIMENTAL

Materials. — Lactulose, cellobiulose, and maltulose were prepared and purified by our procedures[6,7].

Instrumentation. — Proton-noise-decoupled ^{13}C spectra were recorded with JEOL FX60Q* and Brüker WH-180 n.m.r. spectrometers operating at 15.04 and 45.26 MHz, respectively. Each d.i.s. experiment was carried out with a dual coaxial cell previously described[1]. Deuterium oxide was utilized as the internal lock signal and all chemical shifts were measured relative to internal 1,4-dioxane, which was assigned the value of 67.4 p.p.m. Typically, each d.i.s. spectrum required 100 mg of each sample dissolved in 1 mL of D_2O (outer tube) and 100 mg of sample in 1 mL of H_2O (inner tube), respectively. Spectral widths of 1500 Hz, 8k data points, and a recycling time of 3–5 sec were used throughout. For the determination of the contribution of the acyclic keto tautomer, normal spectra were obtained with spectral widths of 4000 Hz, 8k data points and a recycling time of 30 sec. The 4:1 $H_2O–D_2O$ solutions typically contained 200–250 mg of sample and 1 % 1,4-dioxane as a reference.

RESULTS AND DISCUSSION

The d.i.s. technique has proven very useful for elucidating the spectra of mono- and di-saccharides[1a] as well as various modified and substituted carbohydrates[1b]. Table I lists the values for the previously derived d.i.s. parameters used to analyze the ^{13}C spectra of the three keto disaccharides described in this study. To be more general, we have redefined the earlier β^1 and β^6 parameters to β^a (β isotope shift at the anomeric center) and β^p (β isotope shift at a primary carbon atom), respectively, to accommodate the carbon designations for the keto sugars.

Lactulose (**1**), cellobiulose (**2**), and maltulose (**3**) can each potentially exist in solution in five tautomeric forms. For simplicity, we illustrate this point for the

---

*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

TABLE I

^{13}C DIFFERENTIAL ISOTOPE SHIFT PARAMETERS^a (p.p.m.)

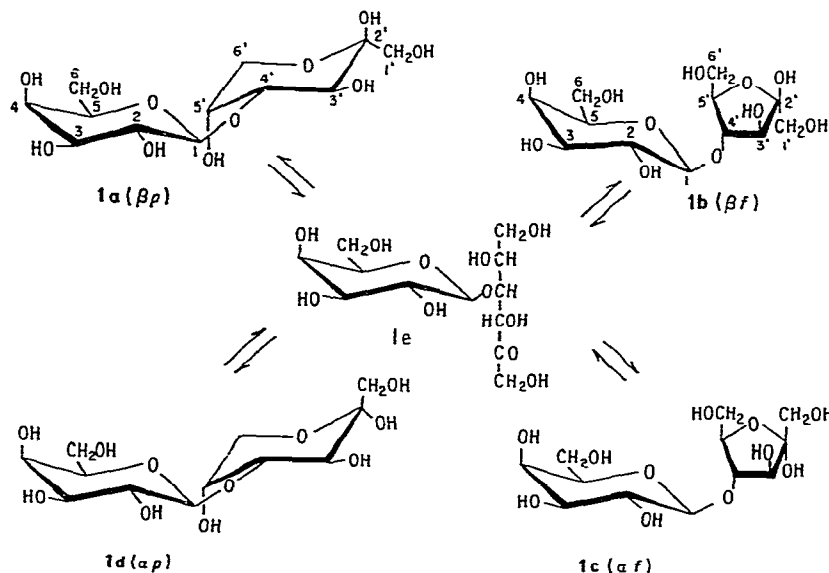| Parameter	Definition
$\beta = 0.14$	Shift induced on ring carbon atoms by directly bonded OD
$\gamma = 0.03$	Shift induced on ring carbon atoms by OD bonded to adjacent carbon
$\beta^a = 0.11$	Shift induced on anomeric carbon atoms by directly bonded OD
$\beta^p = 0.15$	Shift induced on primary carbon atoms (CH$_2$OH) by directly bonded OD

^{a}Calculated from least-squares treatment, see ref. 1.

Fig. 1. Five tautomeric forms of lactulose.

suggested solution equilibrium of the lactulose system, isomers 1a–1e (Fig. 1). To cellobiulose (2a–2e), the structures 1a–1e would be modified by showing the 4 OH group as equatorial rather than axial, and for maltulose 3a–3e the glycosidic linkage would be changed to the α-configuration in addition to epimerization of HO-4. Table II records the proposed ^{13}C resonance assignments; chemical-shift data and d.i.s. values (observed and calculated) for each of the three equilibrated compounds 1, 2, and 3 in water. A fourth isomer, the keto form e, has also been observed in low concentration and is described later in this report.

Lactulose. — To decipher the anomeric composition of these tautomeric mixtures initially, we found it advantageous to establish the identity of the low-field anomeric resonances with the aid of d.i.s. As shown in Fig. 2, the representative areas for each of the observable forms of 1 (a–c) were readily assigned once the isotope

TABLE II

CHEMICAL-SHIFT ASSIGNMENTS AND D.I.S. VALUES[a] OF KETO DISACCHARIDES

Compound	Shifts and d.i.s. in p.p.m.[b,c]											
	C-1	C-2	C-3	C-4	C-5	C-6	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
Lactulose[d]												
β-pyranose (1a)	101.46	71.73	73.65	69.66	76.03	62.06	65.08	98.76	67.16	78.34	67.70	63.88
d.i.s.	~0	0.17	0.21	0.16	0.05	0.15	0.20	0.13	0.16	0.07	0.14	~0
	(0.03)	(0.17)	(0.20)	(0.17)	(0.06)	(0.15)	(0.18)	(0.17)	(0.17)	(0.06)	(0.14)	(0.03)
β-furanose (1b)	103.42	71.73	73.65	69.66	76.03	62.06	65.08	103.05	76.12	84.90	80.82	63.57
d.i.s.	~0						0.20	0.12	0.15	~0	~0	0.17
	(0.03)						(0.18)	(0.17)	(0.17)	(0.03)	(0.03)	(0.15)
α-furanose (1c)	103.85	71.73	73.65	69.66	76.03	62.06	63.88[e]	105.59	81.80	85.98	81.40	63.56[e]
d.i.s.	~0							0.14	0.19	~0	~0	
	(0.03)						(0.18)	(0.17)	(0.17)	(0.03)	(0.03)	(0.15)

Cellobiulose[a]												
β-pyranose (2a)	101.10	74.00	76.68	70.63	76.87	61.76	65.00	99.10	67.08	78.41	67.71	63.88
d.i.s.	~0	0.14	0.22	0.17	0.10	0.12	0.19	0.15	0.15	0.05	0.12	~0
	(0.03)	(0.17)	(0.20)	(0.17)	(0.06)	(0.15)	(0.18)	(0.17)	(0.17)	(0.06)	(0.14)	(0.03)
β-furanose (2b)	103.10	74.00	76.68	70.88	76.87	61.76	63.61[f]	103.20	76.67	84.91	80.92	63.61[f]
d.i.s.	~0			0.17				0.11	0.10	~0	~0	
	(0.03)			(0.17)				(0.17)	(0.17)	(0.03)	(0.03)	(0.03)
α-furanose (2c)	103.51	74.00	76.68	70.63	76.87	61.76	63.61[f]	105.90	81.70	86.21	81.70	63.61[f]
d.i.s.	~0			0.17				0.12	0.19	~0	~0	
	(0.03)			(0.17)				(0.17)	(0.17)	(0.03)	(0.03)	(0.15)
Maltulose[a]												
β-pyranose (3a)	101.48	72.99	74.14	70.86	73.42	61.83	65.08	99.38	68.17	79.15	70.31	64.54
d.i.s.	0.05	0.14	0.21	0.18	0.06	0.17	0.20	0.10	0.15	0.13	0.13	0.03
	(0.03)	(0.17)	(0.20)	(0.17)	(0.06)	(0.15)	(0.18)	(0.17)	(0.17)	(0.06)	(0.14)	(0.03)
β-furanose (3b)	99.35	72.41	74.00	70.68	73.48	61.66	63.75	103.07	76.48	82.36	81.06	63.83
d.i.s.	0.04	0.16	0.21	0.17	0.06	0.16	0.18	0.17	0.13	0.03	0.03	0.13
	(0.03)	(0.17)	(0.20)	(0.17)	(0.06)	(0.15)	(0.18)	(0.17)	(0.17)	(0.03)	(0.03)	(0.15)
α-furanose (3c)	98.85	72.41	74.00	70.68	73.48	61.66	63.75	106.34	81.31	83.33	82.22	62.55
d.i.s.	~0					0.16	0.18	0.15	0.17	0.07	0.05	0.16
	(0.03)					(0.15)	(0.18)	(0.17)	(0.17)	(0.03)	(0.03)	(0.15)

[a]Calculated d.i.s. values appear in parentheses. [b]Shifts are in p.p.m. relative to internal 1,4-dioxane taken as 67.4 p.p.m. [c]Numbers with primes refer to the reducing ring carbon atoms. [d]Spectrum taken at 15 MHz. [e]The d.i.s. values were not extractable because of overlapping peaks. [f]The average d.i.s. value for the four resonances was 0.17. [g]Spectrum taken at 45 MHz (see Fig. 3).
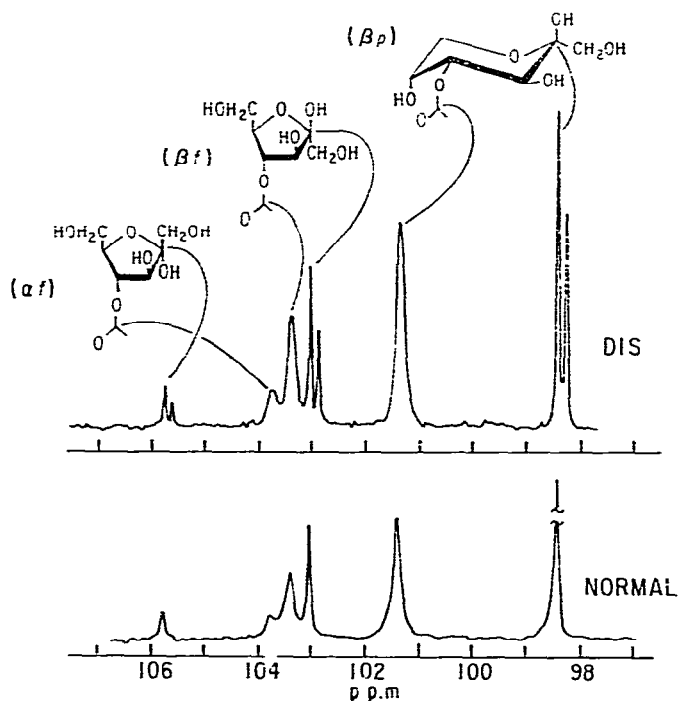
Fig. 2. 15.04-MHz proton-noise-decoupled ^{13}C n.m.r. spectrum of the anomeric region of lactulose:
(A) d.i.s. spectrum; (B) normal spectrum. Displayed spectral width is 500 Hz.

perturbations were evaluated. Fig. 2A (the d.i.s. spectrum) clearly revealed the identity
of the non-OH bearing C-1 anomeric carbon resonances at δ 103.85, 103.42, and
101.46, representing isomers 1c, 1b, and 1a, respectively, as none of these demonstrated
any resolvable isotope shifts. The corresponding C-2' anomeric resonances of 1c, 1b,
and 1a found at δ 105.59, 103.05, and 98.76, respectively, representing the reducing
fructose portion of the foregoing, gave the anticipated upfield shift-patterns as previ-
ously found for the model compound fructose[1]. As noted earlier, the observed d.i.s.
value for C-2' resonances of such ketohexoses as fructose are somewhat smaller than
those calculated, primarily because our derived parameter set utilized aldohexoses
for its basis set[1]. The non-reducing ring carbon atoms 2, 3, and 5 were given tentative
resonance positions based on previous unambiguously established spectral assign-
ments of analogous galactoside ring-systems[3]. Verification of the identity of each
individual absorption was then completed by d.i.s. examination. The resonances
representing carbon atoms 5 and 3 were immediately recognized from their respec-
tively small (0.05 p.p.m.) and large (0.21 p.p.m.) shifts. The C-2 resonance had a
shift of 0.17 p.p.m., as predicted. The remaining C-4 resonance was assigned from
its characteristic high-field position at 69.6 p.p.m. relative to the other ring carbon
resonances, as well as from its consistent d.i.s. value. The evaluation of the positions
and d.i.s. values of the reducing-ring resonances 3', 4', and 5' of the β-pyranose

isomer **1a** was performed similarly. While there was no problem to identify the glycosylated C-4' resonance by both its relatively low-field position and small shift (0.07), the differentiation between C-3' and C-5' was less clear-cut because of the relatively small differences in observed d.i.s. Ultimately, to verify the assignments, we deferred to an earlier, unambiguous isotope-labeling experiment that had made a clear distinction between the C-3' and C-5' resonances in unsubstituted fructose[4]. Our experimentally determined d.i.s. values indicate that the assignments for C-3' and C-5' in **1a** are in accord with those found for fructose[1].

The resonance absorptions examined for forms **1b** and **1c** were almost exclusively restricted to those representing the reducing-ring moieties, as all of the nonreducing ring lines except for that of C-1 were common to all three tautomeric forms. The established ratio of the isomers (obtained from the anomeric region of the spectrum) was conveniently used to assign the remaining sets of resonances to each of the respective tautomers (**1b** and **1c**). Selection of the glycosylated ring carbon atoms C-4' was readily made from their low-field positions (~ 86 p.p.m.) and their zero isotopic shift. Carbon 5 resonances were found at somewhat higher field, but also exhibited essentially zero isotopic shifting. The remaining resonances at the intermediate field positions were attributed to C-3', with a shift of 0.17–0.19 p.p.m. To differentiate between C-6' and C-1' resonances is normally difficult because of the close proximity of both ^{13}C and ^{1}H chemical shift positions and the identity of ^{1}H coupling multiplicities. However, the difference in d.i.s. can often help to distinguish these absorptions from one another. As may be seen in Table II, the carbon atom identified as C-1' for isomer **1b** corresponds to the lower-field resonance (65.08 vs. 63.57 p.p.m.) having the larger of the two isotopic shifts (as predicted from $\beta + \gamma$ contributions). Unfortunately, resonance overlaps preclude recognition of d.i.s. values and differentiation of these corresponding absorptions in **1c**.

Cellobiulose. — The spectral assignments of cellobiulose (**2**) were determined in much the same manner as those of lactulose (**1**). As cellobiulose is a glucosyl disaccharide, well established β-glucoside shift-positions were used as preliminary assignments for the non-reducing ring carbon atoms. As with lactulose (**1**), the well dispersed, low-field anomeric region of the spectrum served to evaluate the ratio of the three isomeric forms **2a–c**. With the exception of the C-4' resonance of isomer **2c**, we have found excellent correspondence between the relative positional assignments for cellobiulose (**2**) and lactulose (**1**). Differentiation of C-1' and C-6' absorptions for **2b** and **c** could not be accomplished because of the coincidence of these shifts.

Maltulose. — Unlike the spectra of sugars **1** and **2**, that of maltulose (**3**) does not exhibit the commonality of carbon resonances 2–6 among its three tautomers **a**, **b**, and **c**. However, this added complication was alleviated by examination of the spectrum at a higher field. Fig. 3 illustrates the well resolved ^{13}C d.i.s. spectrum of maltulose at 45.6 MHz. The spectral assignments relating to structures **3a–c** are shown in the accompanying figure depicting the tautomeric equilibrium, and the corresponding shift-values are listed in Table II. With the exception of four newly established resonance shift-positions (two alternate and two clarified), our d.i.s.
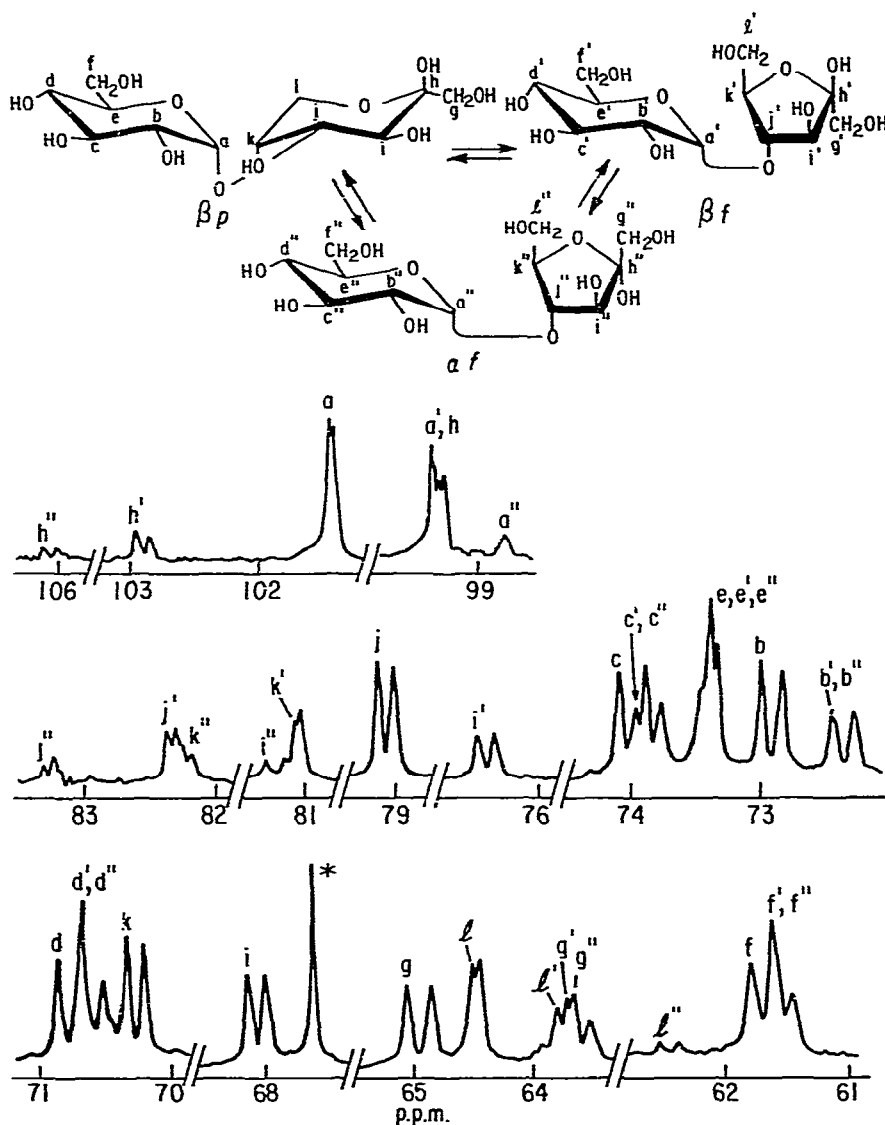
Fig. 3. 45.6-MHz proton-noise-decoupled, natural-abundance ^{13}C d.i.s. spectrum of matulose. Spectral width = 3,000 Hz, displayed width 2,400 Hz, number of transients = 3,300.

assignments correspond to those made in the initial report of the ^{13}C spectrum of maltulose of Jarrell et al.[5]. All of the relative positions of the designated resonances representing the non-reducing α-glucoside ring carbon atoms in isomers 3a–e are consistent with our earlier report on methyl α-D-glucopyranoside[1]. The d.i.s. shifts for carbon resonances at δ 81.31 (0.17) and 83.33 (0.07) correspond to C-3' and C-4' of the α-furanose 3c, respectively. This constitutes an interchange of the position assignment made earlier[5]. Furthermore, we have also been able to clarify the previous

designation[5] for carbon atoms 1' and 6' of isomers **3a** and **b**, as the resonances for C-1' have slightly larger d.i.s. values than those corresponding to C-6' (1' = 0.18, 6' = 0.15).

GENERAL OBSERVATIONS

An examination of the d.i.s. values for the C-4' resonances representing the site of glycosylation of the fructopyranose group in **1a**, **2a**, and **3a** revealed a possible correlation of d.i.s. values with the stereochemistry of the glycosidic linkage. In particular, we observe that the fructopyranose forms of lactulose (**1a**) and cellobiulose (**2a**) show d.i.s. values of 0.03 p.p.m. for the C-4' resonances, consistent with those previously noted in β-(1→4)-linked disaccharides[1]. In contrast, the C-4' resonance of the α-linked β-fructopyranose form (**3a**) of maltulose exhibited a significantly larger value of 0.13 p.p.m. (twice the predicted value, see Table III). To verify the generality of this phenomenon, we examined the d.i.s. shifts of the glycosylated carbon atoms of ten other disaccharides whose glycosidic linkages were either 1→2, 1→3, or 1→4. The data in Table III show that the two anomeric forms of the α-linked disaccharides nigerose (3-O-α-D-glucopyranosyl-D-glucopyranose) and maltose (4-O-

TABLE III

D.I.S. VALUES (p.p.m.) FOR GLYCOSYLATED CARBON RESONANCES IN α AND β LINKED PYRANOSE DI-SACCHARIDES

Compound anomer	Glycosylated carbon atom	Linkage	Observed d.i.s.	Calc. d.i.s.
Lactose				
α	4	β	0.03	0.03
β	4	β	0.03	0.03
Cellobiose				
α	4	β	0.03	0.03
β	4	β	0.03	0.03
Maltose				
α	4	α	0.10	0.03
β	4	α	0.10	0.03
Nigerose				
α	3	α	0.12	0.06
β	3	α	0.13	0.06
Sophorose				
α	2	β	0.06	0.06
β	2	β	0.06	0.06
Maltulose				
β	4	α	0.13	0.06
Lactulose				
β	4	β	0.07	0.06
Cellobiulose				
β	4	β	0.05	0.06

α-D-glucopyranosyl-D-glucopyranose) afford the same large d.i.s. values (0.10–0.13) for C-3′ and C-4′ as found for maltulose. However, the β-(1→2)-linked sugar sophorose (2-O-β-D-glucopyranosyl-D-glucopyranose) shows shifts consistent with the other β-linked series. It is evident from these data that the d.i.s. for glycosylated carbon resonances in pyranose ring-systems* is diagnostic for defining the stereochemistry of the non-reducing ring residue.

The unusually large perturbing shift exhibited by the glycosylated carbon resonances of the α-linked pyranose disaccharides could be related not only to isotope contributions but also to small differences in the conformational distribution of these molecules when placed in the dual opposing solvent-environments (D_2O vs H_2O). Because of the axial stereochemistry at C-1, intramolecular ring–ring interactions are maximized for α-linked disaccharides, whose non-reducing ring assumes the favored $^4C_1$ configuration. Under the influence of different solvents (H_2O vs D_2O), a delicate balance between the relative amounts of ring–ring and ring–solvent interactions could produce differences in conformational equilibria that would ultimately translate into chemical-shift perturbations. In contrast, the equatorial C-1 linkage of the β-linked disaccharides holds the reducing and non-reducing rings far apart on the average, rendering them relatively non-interactive. A similar argument has been brought out for interpretation of optical rotation behavior, which suggests that the β-linked cellobiose in water solution stays close to its conformation in the crystal, except for some fluctuations from bond oscillation and rotation. In contrast, maltose, the α-linked disaccharide, partially populates its energy map differently because the water molecules compete effectively with sugar hydroxyl groups for hydrogen-bonding sites[8].

A closer examination of the normal ^{13}C spectrum of each of the three keto disaccharides 1, 2, and 3 revealed the presence of a carbonyl resonance at 113 p.p.m. corresponding to a small proportion of the acyclic keto forms 1e, 2e, and 3e**. A similar observation has recently been made in studies of the solution chemistry of fructose[9a] and fructose 1,6-biphosphate[9b].

Estimates of the amount of keto form present in each of these sugars was provided by integration of the carbonyl resonance and its comparison with the area of the anomeric resonances. Because of the uncertainty in the relaxation time of the carbonyl resonance, the repetition time for these experiments was set at 30 sec, with a pulse width of only 50°. As these times may not be long enough to achieve full relaxation for the carbonyl resonance, it is suggested that the percentages reported here be considered as minimum values (Table IV). In lactulose, the proportion of keto form increases with increasing temperature. These observations are in accord with those reported for fructose[9a]. In all three sugars, the proportion of pyranose

---

*This correlation breaks down for the fructofuranose reducing ring-systems whereby all of the glycosylated carbon resonances exhibit small (0.03–0.06 p.p.m.) shift values, as anticipated.

**Although other peaks representing the carbon atoms of this structure were evident in the upfield portion of the spectrum, their overlap with the main component peaks made their quantification and identification impossible.

TABLE IV

VARIATION IN THE PERCENT DISTRIBUTION OF TAUTOMERIC FORMS OF KETO DISACCHARIDES WITH TEMPERATURE

Compound	Percent composition		
	Temperature 25°	58°	73°
Lactulose			
β-p (1a)	61.5	54.7	52.1
β-f (1b)	29.3	31.6	35.5
α-f (1c)	7.6	11.2	12.4
keto form (1e)	1.6 ±0.5	2.5 ±0.5	nd[a]
Cellobiulose			
β-p (2a)	61.3	55.4	
β-f (2b)	28.8	32.5	
α-f (2c)	9.9	9.9	
keto form (2e)		2.2 ±0.5	
Maltulose			
β-p (3a)	64.0 (61.5)[b]	55.0 (42.9)[b]	
β-f (3b)	22.4 (30.7)[b]	29.6 (42.9)[b]	
α-f (3c)	12.1 (7.6)[b]	14.3 (14.3)[b]	
keto form (3e)	1.5 ±0.5	2.1 ±0.5	
Fructose			
β-p	75.0[c]	66.0[c]	
β-f	21.0[c]	28.0[c]	
α-f	4.0[c]	6.0[c]	

[a]Not determined because of excessive sample decomposition at this higher temperature over the long spectral accumulation times required. [b]Ref. 5; these values were derived from studies at 32 and 60°, respectively. [c]Ref. 4; these values were derived from studies at 27 and 55°, respectively.

form decreases at higher temperatures. However, we do not observe the large increase in the β-furanose tautomer 3b concentration described earlier[5] for maltulose, but only about a 7% increase. We do, however, observe a small decrease (~10%) in 3a, a 2% increase in 3c, and a 1% increase in the keto form 3e. Similar results were obtained for lactulose and cellobiulose. These present data indicate that the stability of the β-furanose form is similar to that reported for fructose[9a] (Table IV) in spite of the substitution of the former at O-4'. One difference, which suggests a perturbation brought about by glycosylation at O-4', is the two to three fold higher concentration (relative to fructose[9a]) of the α-furanose forms 1c, 2c, and 3c at all temperatures. Unlike the previous study of maltulose[5], we observe a higher concentration of the β-pyranose form 3a relative to 3b furanose form at 58°. At present we cannot explain this discrepancy.

REFERENCES

1 (a) P. E. PFEFFER, K. M. VALENTINE, AND F. W. PARRISH, J. Am. Chem. Soc., 101 (1979) 1265–1274; (b) P. E. PFEFFER, F. W. PARRISH, AND J. UNRUH, Carbohydr. Res., 84 (1980) 13–23; (c) K. M. VALENTINE, L. W. DONER, AND P. E. PFEFFER, Carbohydr. Res., 96 (1981) 293–298.
2 A. J. JONES, P. HANISCH, AND A. K. McPHAIL, Aust. J. Chem., 32 (1979) 2763–2766.
3 (a) P. A. J. GORIN AND M. MAZUREK, Can. J. Chem., 53 (1975) 1212–1223; (b) T. E. WALKER, R. E. LONDON, T. W. WHALEY, R. BARKER, AND N. A. MATWIYOFF, J. Am. Chem. Soc., 98 (1976) 5807–5813.
4 S. J. ANGYAL AND G. S. BETHELL, Aust. J. Chem., 29 (1976) 1249–1265.
5 H. C. JARRELL, T. F. CONWAY, P. MOYNA, AND I. C. P. SMITH, Carbohydr. Res., 76 (1979) 45–57.
6 K. B. HICKS AND F. W. PARRISH, Carbohydr. Res., 82 (1980) 393–397.
7 K. B. HICKS, E. V. SYMANSKI, AND P. E. PFEFFER, unpublished results.
8 D. A. REES, AND P. J. C. SMITH, J. Chem. Soc., Perkin Trans. 2, (1977) 191–201.
9 (a) W. FUNCKE, C. VON SONNTAG, Carbohydr. Res., 75 (1979) 305–309; (b) C. F. MADELFORT, R. K. GUPTA, AND I. A. ROSE, Biochemistry, 15 (1976) 2178–2185.