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STABILIZATION OF THE β-FURANOSE FORM, AND KINETICS OF THE TAUTOMERIZATION OF D-FRUCTOSE IN DIMETHYL SULFOXIDE

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ABSTRACT

An explanation is offered for the marked increase observed in the relative stability of the β-furanose form of D-fructose in dimethyl sulfoxide as compared to that in water. Evidence obtained at 400 MHz, over a range of temperatures, indicates that HO-3 and HO-4 are hydrogen-bonded to primary hydroxyl groups HO-6 and HO-1, respectively. It is proposed that this intramolecular association between pairs of secondary and primary hydroxyl groups is a major source of the enhanced stabilization of the β-furanose form relative to the other tautomeric forms of the ketose. The kinetics of this equilibrium have been studied by ^{1}H saturation-transfer n.m.r. spectroscopy. As the acyclic form, through which the interconversions are believed to occur, was not detected, the measured rate constants represent apparent rate constants. Their magnitudes, which are a measure of the direction towards equilibrium of the four tautomers of D-fructose, are in accord with the relative proportions of these forms as measured from the spectra.

INTRODUCTION

Several sugars and derivatives exhibit[1–3] a higher proportion of furanose forms in dimethyl sulfoxide than in water. The change is particularly striking[3] for D-fructose. According to ^{1}H- and ^{13}C-n.m.r. spectroscopic observations on this ketose in water[3–6], the β-pyranose, (**1**), β-furanose (**2**), α-furanose (**3**), and α-pyranose (**4**) tautomers comprise a 6:3:1:trace mixture, whereas their equilibrium ratio in dimethyl sulfoxide[3] is 1:3:1.3:trace. It has been suggested[3] that, in the latter solvent, there is preferential stabilization of the β-furanose through intramolecular hydrogen-bonding between HO-1 and HO-4. This proposal is now modified in the light of more extensive ^{1}H-n.m.r. observations. Also, an analysis is presented of the kinetics of interconversions between the tautomeric forms of D-fructose in dimethyl sulfoxide, through application of the "^{1}H-saturation transfer" technique[7,8].

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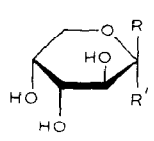
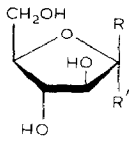




1 R = OH, R′ = CH_2OH 2 R = OH, R′ = CH_2OH

4 R = CH_2OH, R′ = OH 3 R = CH_2OH, R′ = OH

EXPERIMENTAL

^{1}H-N.m.r. spectra were recorded with a Varian XL-200 or a Bruker WH-400 spectrometer. An equilibrated 0.2M solution of D-fructose in $(CD_3)_2SO$ was obtained by storage for several weeks. Before examination, the solution was deoxygenated with a stream of dry nitrogen. The 400-MHz ^{1}H-n.m.r. spectrum was recorded at 20° and 40°.

Proton saturation-transfer experiments were carried out at 40°, at a frequency of 200 MHz: the effects on the anomeric hydroxyl proton (HO-2) signals of any three of the tautomers were observed while HO-2 of the fourth tautomer was saturated by gated decoupling, using a 90° pulse and delays of ≥20 s between pulses. The irradiation power was the minimum required for effective saturation of the irradiated nucleus. At least 10 experiments were performed with the saturating field on and/or gated off-resonance. The saturation-transfer effects listed in Table I, together with standard deviations, were calculated from eq. *2* (see below) by use of the printout intensities.

The recovery of magnetization of the HO-2 signal of any given tautomer upon complete saturation of HO-2 of another tautomer was observed by the two-pulse IRFT technique; the observation 90°-pulse was 8.0 μs, the relaxation delay between

TABLE I

SATURATION-TRANSFER EFFECTS ON THREE SITES WHILE SATURATING A PROTON OF THE FOURTH SITE IN THE FOUR-SITE EXCHANGING SYSTEM OF D-FRUCTOSE IN DIMETHYL SULFOXIDE[a,b]

Proton irradiated	*Proton observed*			
	HO-2(α-p),(i)	*HO-2(β-p),(j)*	*HO-2(β-f),(u)*	*HO-2(α-f),(v)*
HO-2(*α-p*),(i)	—	0.243 (±0.005)	0.232 (±0.004)	0.225 (±0.008)
HO-2(*β-p*),(j)	0.267 (±0.003)[c]	—	0.334 (±0.009)	0.259 (±0.012)
HO-2(*β-f*),(u)	0.348 (±0.04)	0.477 (±0.018)	—	0.491 (±0.020)
HO-2(*α-f*),(v)	0.328 (±0.038)	0.266 (±0.008)	0.278 (±0.004)	—

[a]Negative values. [b]The equilibrium intensities are 8.216 ±0.557, 44.045 ±0.045, 104.297 ±0.977, and 71.919 ±0.971 for sites HO-2(*α-p*), HO-2(*β-p*), HO-2(*β-f*), and HO-2(*α-f*), respectively. [c]Values in parentheses are standard deviations of the measurements.

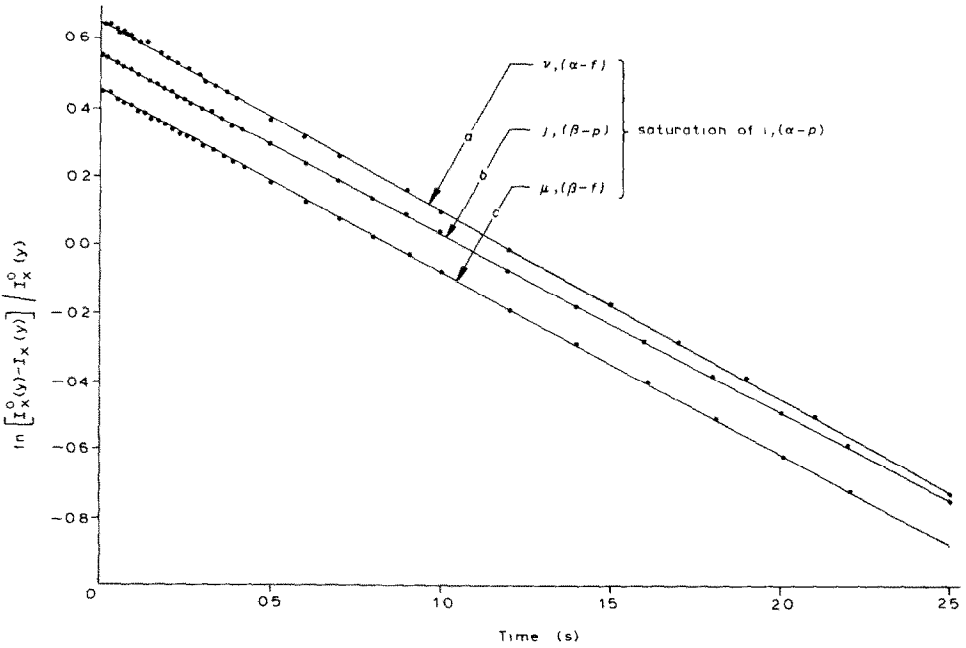


Fig. 1. Decay of magnetization of tautomeric forms of D-fructose as a function of time (t), measured by ^{1}H saturation-transfer n.m.r. spectroscopy: (*a*) α-furanose, x = v, y = i; (*b*) β-pyranose, x = j, y = i; (*c*) β-furanose, x = u, y = i; upon continuous saturation of the α-pyranose form, y = i. The vertical scales of (*b*) and (*c*) are displaced downwards by one unit and two units, respectively.

the two pulses was ≥20 s, and 25–30 spectra having different τ values (0.01–3 s) were acquired automatically. At least four spectra with $\tau \geq 20$ s [defined as $I_j^\circ(i)$ spectra] were obtained at the beginning and the end of each experiment. To avoid systematic errors, the various τ values were arrayed in each run by utilizing the XL-200 spectrometer program. Typical semi-logarithmic plots of the decay of I_z as a function of time τ are shown in Fig. 1 for the β-pyranose, α-furanose, and β-furanose forms upon continuous saturation of the α-pyranose form of D-fructose.

RESULTS AND DISCUSSION

The 400-MHz hydroxyl-proton n.m.r. spectrum of β-D-fructofuranose (**2**) *in dimethyl sulfoxide.* — The signal at δ 5.15 in the 400-MHz ^{1}H-n.m.r. spectrum of an equilibrated solution of D-fructose in $(CD_3)_2SO$ at 20° (Fig. 2A) is an asymmetric multiplet. In the 100-MHz spectrum reported earlier[3], the corresponding signal appeared to be a doublet of doublets, and was attributed to HO-1 of the β-furanose (**2**). By contrast, when the well-resolved HO-2 signal of **2** in Fig. 2A was compared in intensity with that of the multiplet, it was evident that the latter signal arises from two hydroxyl groups (rather than one) of the same tautomer. This conclusion was confirmed when, at 40° (Fig. 2B), the multiplet was clearly separated into two

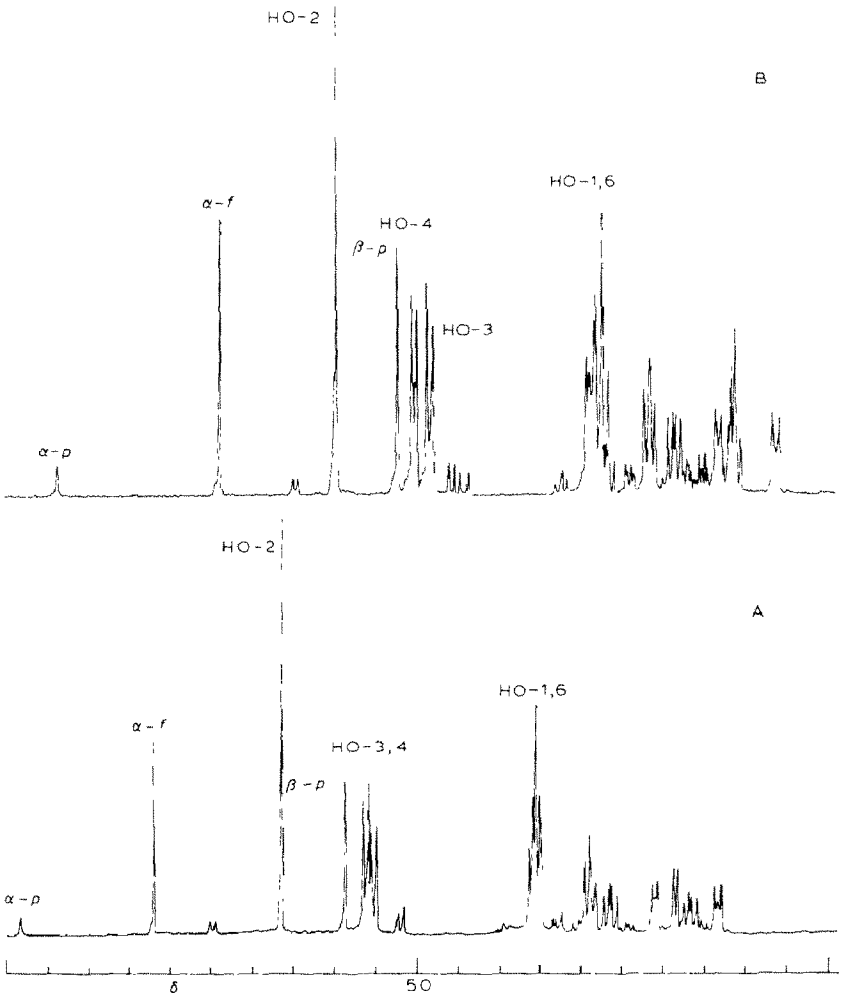
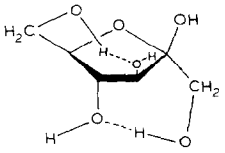


Fig. 2. Partial ^{1}H-n.m.r. spectrum (400 MHz) of an equilibrated solution of D-fructose in $(CD_3)_2SO$ at (A) 20° and (B) 40°. Signals upfield of δ 4.0 are not shown. Signals HO-1,2,3,4,6 are due to β-D-fructofuranose, signals designated α-*p*, α-*f*, and β-*p* are those of HO-2 of the α-pyranose, α-furanose, and β-pyranose forms, respectively.

doublets, at δ 5.01 and 4.97, having spacings of 5.47 and 5.77 Hz, respectively. These signals must be attributed, therefore, to HO-3 and HO-4 of the β-furanose (**2**). As they absorb at relatively low field, and their chemical shifts decrease notably with an increase in temperature, they are likely[9,10] to be hydrogen-bonded groups, although not to each other because of their *trans*-configuration. A plausible arrangement could involve intramolecular hydrogen-bonding of HO-3 with HO-6, and of HO-4 with HO-1, as shown in **5***. The characteristics of the HO-1 and HO-6

*This representation of H-bonding incorporates only one possible combination of H-donor and H-acceptor (see refs. 9 and 10).

signals are in agreement with this suggestion. Based on integration measurements, it appears that these two signals overlap with each other (as well as with an HO signal of the α-furanose (**3**) to form the multiplet at δ 4.75 (Fig. 2A, 20°), which is at low field relative to the ^{1}H-resonances of non-hydrogen-bonded primary hydroxyl groups[1-3,9,11]. With an increase in the temperature to 40° (Fig. 2B), as expected, the corresponding signals are shifted upfield to δ 4.57 and 4.55.



5

Kinetics of the tautomeric equilibrium (mutarotation). — The tautomeric equilibrium between four interconverting forms of D-fructose in solution, namely, α-pyranose (α-*p*), β-pyranose (β-*p*), α-furanose (α-*f*), and β-furanose (β-*f*), will be treated as a four-site exchange problem (Fig. 3). In this system, the hydrogen of HO-2 of D-fructose is "transferred" to four magnetically nonequivalent sites during the complex tautomerization process, which is characterized in Fig. 3 by various first-order rate constants. It may be noted that this model is a simplification of the widely accepted working hypothesis[12], according to which the interconversion reactions occur through an acyclic intermediate. Unfortunately, the acyclic form, and/or its hydrated analogs, can be detected only at high concentrations[5,13], at which intermolecular dipolar interactions might interfere with the interpretation of the experimental data. Therefore, as a relatively dilute solution is described here, the simpler kinetic scheme is adopted, with the notion that the measured rate constants represent apparent rate constants.

The time dependence of exchanging spin systems can be described[8] by a set of differential equations of the form:

$$\frac{dI_j}{dt} = -(k_{ji} + k_{ju} + k_{jv} + R_{1,j})(I_j - I_j^\circ) + k_{ij}(I_i - I_i^\circ) + k_{uj}(I_u - I_u^\circ) + k_{vj}(I_v - I_v^\circ), \qquad (1)$$

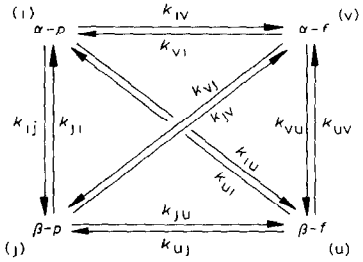


Fig. 3. Tautomeric equilibrium between four interconverting forms of D-fructose in solution (*p* – pyranose, *f* – furanose).

where $R_{1,j}$ is the spin–lattice relaxation rate for site j, k_{ji} is the first-order rate constant for exchange from site j to site i, I_j is the n.m.r. intensity of site j at time t, and I_j° is the equilibrium intensity. Analogous equations may be written for sites i, u, and v.

In order to calculate the twelve rate constants, two types of experiments were performed. The first experiment was analogous to a nuclear Overhauser enhancement (n.O.e.) experiment, in which a given site is saturated and the steady-state intensities (relative to off-resonance equilibrium intensities) at the other sites are measured. When there is exchange, saturation of one site will decrease the intensities at the other sites, provided that rates of exchange and spin–lattice relaxation rates are comparable. Therefore, the observed n.O.e. values are negative. These are defined as

$$f_j(i) = \frac{I_j(i) - I_j^\circ}{I_j^\circ}. \tag{2}$$

Here, $I_j(i)$ is the intensity of site j upon saturation of site i, and I_j° is its equilibrium intensity without saturating irradiation. Twelve such negative values are measured and summarized in Table I.

From Eqs. *1* and *2*, it follows that

$$f_j(i) = \frac{k_{uj}f_u(i)P_u + k_{vi}f_v(i)P_v - k_{ij}P_i}{P_j(k_{ji} + k_{ju} + k_{jv} + R_{1,j})}, \tag{3}$$

where P_i, P_j, P_u, and P_v are the populations at each respective site. Analogous equations may be written for the twelve negative saturation-transfer values by permutation of the indices.

The second experiment was a measurement of the apparent, nonselective spin–lattice relaxation rate of one site when another site is irradiated. If sites j, u, and v have the same apparent relaxation rate when site i is saturated, it is adequate to measure relaxation rates for these sites while saturating site i only[14]. The suitability of this approximation can be seen in Fig. 1, in which the decay of magnetization vectors of sites j(β-p), u(β-f), and v(α-f) upon saturation of site i(α-p) are given as a function of time τ. The slopes of these semi-logarithmic plots are all equal to $0.53 \pm 0.03\ s^{-1}$. Since $I_j(i)$ [and $I_u(i)$, $I_v(i)$] approaches $I_j^\circ(i)$ [and $I_u^\circ(i)$, $I_v^\circ(i)$] as a simple exponential, it follows that the slope of the plot in Fig. 1 is

$$M_j(i) = -(k_{ji} + k_{ju} + k_{jv} + R_{1,j}) + k_{uj}P_u/P_j + k_{vi}P_v/P_j, \tag{4}$$

and similarly for sites u and v.

Equations analogous to *4* may be expressed in terms of the irradiation of sites j, u, and v, one at a time, and observing the decay of the magnetization of the sites if the above approximation holds. However, plots of the decay of the magnetization

as a function of time in some experiments, *e.g.*, irradiating v(α-*f*) while observing u(β-*f*), showed a slight curvature, indicative of the presence of cross-relaxation effects of unknown magnitude. Nevertheless, the total number of equations derivable from all of the relaxation experiments is redundant relative to the number of unknowns (24 equations and only 15 unknown rate constants and relaxation rates). Therefore, it should be feasible to base the present calculations on Eq. *4*, when cross-relaxation effects appear to be insignificant and the sites are characterized by similar, apparent relaxation rates. In this case, the quantities $[I_j(i) - I_j^\circ]/[I_v(i) - I_v^\circ]$, $[I_u(i) - I_u^\circ]/[I_j(i) - I_j^\circ]$, and $[I_u(i) - I_u^\circ]/[I_v(i) - I_v^\circ]$ which appear in Eq. *1* are time-independent and can be set equal to P_j/P_v, P_u/P_j, and P_u/P_v, respectively.

Solution of simultaneous Eqs. *3* and *4* gives rate constants in the form

$$k_{ij} = M_j(i) \frac{\begin{vmatrix} f_j(i) - f_u(i) & f_j(i) - f_v(i) & f_j(i) \\ f_i(u) + 1 & f_j(u) - f_v(u) & f_j(u) \\ f_j(v) - f_u(v) & f_j(v) + 1 & f_j(v) \end{vmatrix}}{\begin{vmatrix} f_j(i) - f_u(i) & f_j(i) - f_v(i) & 1 \\ f_j(u) + 1 & f_j(i) - f_v(u) & -f_i(u) \\ f_j(v) - f_u(v) & f_j(v) + 1 & -f_i(v) \end{vmatrix}} \frac{P_j}{P_i} \quad (5)$$

The calculated rate constants and relaxation rates are summarized in Table II. Although the values in Table II represent apparent rate constants, their magnitude is a measure of the direction of equilibrium among the various forms of D-fructose. All the pairs of rate constants in Table II, *e.g.*, k_{iu}, k_{ui}, *etc.*, favor the formation of the β-furanose form, and disfavor the α-pyranose, in accord with the experimentally determined proportions of these two forms in solution in dimethyl sulfoxide. Moreover, the shift in the equilibrium between the β-pyranose (**1**) and α-furanose (**3**) in favor of **3** agrees with the fact that the α-furanose form is more prominent than **1** at equilibrium.

In conclusion, the present study provides evidence that the preponderance of β-D-fructofuranose in dimethyl sulfoxide may be attributed (at least, in part) to an increase in its relative stability, promoted by intramolecular hydrogen-bonding between two pairs of primary and secondary hydroxyl groups, as represented by **5**. Furthermore, the application of the ^{1}H-n.m.r. saturation-transfer technique to the mixture of tautomeric forms of D-fructose has permitted the determination of apparent rate constants that characterize the equilibration of the four species detected. As acyclic forms of D-fructose were not observed under our experimental conditions, it was not possible to determine ring-opening and ring-closing rate constants as well. However, this may be feasible by utilizing ^{13}C-enriched D-fructose because, as noted above, an acyclic form has been observed in the ^{13}C-spectrum at high concentration (~3% at 80° in a 3.7M solution of D-fructose).

TABLE II

APPARENT RATE CONSTANTS (k)[a] AND RELAXATION RATES (R)[a] FOR D-FRUCTOSE IN DIMETHYL SULFOXIDE AT 40°[b]

k_{ij}	k_{ji}	k_{iu}	k_{ui}	k_{iv}	k_{vi}	k_{ju}	k_{uj}	k_{jv}	k_{vj}	k_{uv}	k_{vu}	$R_{i,i}$	$R_{i,j}$	$R_{i,u}$	$R_{i,v}$
0.32 (±0.01)	0 027 (±0.009)	1.53 (±0.05)	0.014 (±0.01)	1.00 (±0 08)	0.026 (±0.04)	0.55 (±0.04)	0.19 (±0.02)	0.16 (±0.06)	0.09 (±0.07)	0.19 (±0.01)	0.32 (±0.01)	0.25 (±0.04)	0 49 (±0.03)	0.42 (±0 05)	0.52 (±0.06)

[a]Units of s^{-1}. [b]Values of $P_j/P_v = 0.612$, $P_u/P_j = 2.37$, and $P_u/P_v = 1.45$ were used in the calculation. [c]Values in parentheses represent the propagated error of the experimental quantities used for the calculations

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