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THE CONFORMATIONAL PROPERTIES OF SUCROSE IN AQUEOUS SOLUTION: INTRAMOLECULAR HYDROGEN-BONDING

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ABSTRACT

A detailed analysis is presented of the nuclear (^1H and ^{13}C) magnetic resonance (n.m.r.) properties of sucrose, using both D_2O and dimethyl sulfoxide-d_6 as solvents, based on measurements of coupling constants, chemical shifts, T_1 relaxation times, and nuclear Overhauser enhancements. Molecular modelling (HSEA calculations) suggests a strong conformational preference about the glycosidic linkages that is near to that for sucrose in the crystalline state, and this conformational rigidity is fully supported by the n.m.r. data, in terms of lack of influence of changes in concentration and temperature on the relevant n.m.r. parameters. The restricted rotation for the 1-hydroxymethyl group of the fructose residue is related to the persistence of the intramolecular hydrogen-bond between O-1^f and O-2^g. The presence of this bond was established for solutions in $(CD_3)_2SO$ by the observation of isotopic chemica-shifts on partial deuteration of the hydroxyl groups. The orientation of the 6-hydroxyl methyl group of the fructose residue is not that present in the crystalline state but, in $(CD_3)_2SO$, it may be intramolecularly hydrogen-bonded, as was demonstrated by titration of the hydroxyl groups with CD_3OD. Observations are made regarding hydrophobic topographies common to sucrose, saccharin, and 1-chloro-1-deoxy-sucrose, which may have a bearing on sweetness.

INTRODUCTION

Since our preliminary communication[1] on the n.m.r. (^1H and ^{13}C) spectra of sucrose in D_2O and $(CD_3)_2SO$, publications have appeared on both laser–Raman[2,3] and X-ray diffraction[4] studies of sucrose in aqueous solutions, wherein it was concluded that, in dilute solution, sucrose lacks intramolecular hydrogen-bonds but, "as the concentration is increased, the bringing together of molecules is accompanied by a twisting around the glycosidic linkage, C-1-O-C-2', that leads to the form of the sucrose molecule found in the crystal, including two intramolecular hydrogen-bonds." Our results are not in accord with these conclusions and we now provide experimental

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data which support this contention. Indeed, our results strongly support the contention that sucrose exists in aqueous solution in a form wherein the OH-1[f] to O-2[g] hydrogen-bond (which exists in the crystal lattice[5]) is maintained even in dilute solution. Furthermore, our n.m.r. study strongly supports the contention, based on HSEA molecular modelling, that sucrose is basically a rather rigid molecule. Only slight flexing of the furanoid ring is apparent, along with different degrees of freedom of rotation about the hydroxymethyl to carbon bonds. A comparison of the n.m.r. data for sucrose in $(CD_3)_2SO$ with those in D_2O helped to clarify the state of sucrose when dissolved in water.

DISCUSSION OF RESULTS

Casu and co-workers[6] provided a detailed consideration of the association of carbohydrates with dimethyl sulfoxide through hydrogen bonding and the possible influences on conformational equilibria which may arise from the balancing of non-bonding and hydrogen-bonding energies. Although Symons and co-workers[7] have demonstrated that, at low temperatures and appropriate pH, the hydroxyl-proton resonance spectra of carbohydrates can be resolved, the quality of the spectra achieved are not adequate for the examination of intramolecular hydrogen-bonds that may exist[4] for sucrose in aqueous solution.

TABLE I

CHEMICAL SHIFTS AND COUPLING CONSTANTS [δ (J, Hz)] FOR SUCROSE IN D_2O AND $(CD_3)_2SO$

^1H Data[a]	*H-1^g*	*H-2^g*	*H-3^g*	*H-4^g*	*H-5^g*	*H-6^g–H-6'^{g}*
D_2O	5.664(3.6)	3.808(9.8)	4.010(9.7)	3.719(9.3)	4.110	4.08–4.01
$(CD_3)_2SO$	5.586(3.5)	3.589(10.0)	3.876(9.7)	3.520(9.4)	4.054(2 0,4.5)	4.00–3.92
OH-Resonances		5.378(5.6)	5.093(4.9)	5.080(5.6)		4.731(5.8)
	H-1^f		*H-3^f*	*H-4^f*	*H-5^f*	*H-6^f–H-6'^{f}*
D_2O	3.930		4.465(8.8)	4.300(8.6)	4.142	4.08–4.01
$(CD_3)_2SO$	3.818		4.283(8.1)	4.178(7.6)	3.995	4.00–3.92
OH-Resonances	5.137(6.3)		4.816(8.0)	5.518(5.3)		4.711(5.6)

^{13}C Data[b]	*C-1^g*	*C-2^g*	*C-3^g*	*C-4^g*	*C-5^g*	*C-6^g*
D_2O	92.97(169)	71.88(144)	73.41(143)	70.08(143)	73.21(141)	61.02(143)
$(CD_3)_2SO$	92.97(168)	72.65(141)	74.00(140)	70.98(141)	73.82(140)	61.66(140)
	C-1^f	*C-2^f*	*C-3^f*	*C-4^f*	*C-5^f*	*C-6^f*
D_2O	62.28(143)	104.49(4.5)[c]	77.34(145)	74.88(147)	82.16(149)	63.16(142)
$(CD_3)_2SO$	63.28(142)	105.03	78.33(144)	75.44(146)	83.48(148)	63.12(141)

[a]Data obtained at 400 MHz at 310 K for 0.1M solutions; 1% of acetone (δ 2.480) used as internal reference. δ Value (J value; observed, first-order coupling-constants). [b]Data obtained at 100 MHz at 310 K for 0.3M solutions; 2% of 1,4-dioxane (67.40 p.p.m.) used as internal reference. δ Value (^1J value). [c] $^3J_{C\text{-}2^f,H\text{-}1^g}$ value.

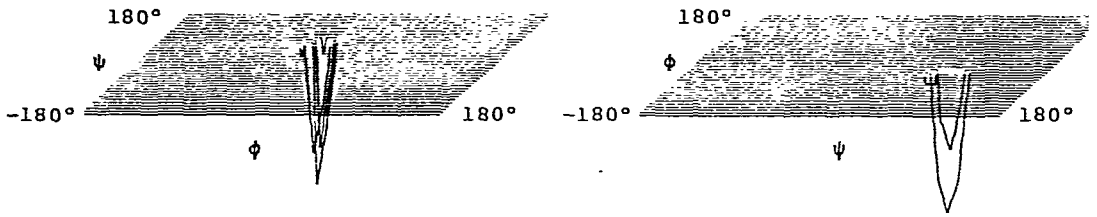


Fig. 1. Computer-drawn energy surface for sucrose, as derived by HSEA calculations over the ranges −180° to 180° for both the ϕ^{H} and ψ^{C-1f} torsion angles, in order to illustrate that the molecule is expected to reside largely in a narrow energy-well as conformers not differing by more than 2.5 kcal/mol.

As previously mentioned[1], the small changes in chemical shifts (see Table I) for the ^{13}C- and ^{1}H-atoms of sucrose, which arise from changing the solvent from D_2O to $(CD_3)_2SO$, result largely from general solvent effects, with sucrose maintaining the same conformation in both solvents. This apparent rigidity of the sucrose molecule is well supported by HSEA calculations[8,9]. As seen in Fig. 1, these calculations set sucrose in a deep, rather narrow energy-well with the most stable conformers predicted to be near those established for sucrose in the crystalline state by neutron diffraction[5]. For those conformations which differ by <2.5 kcal/mol, the range of torsion angles for the atoms about the glycosidic bonds are −20° to −10° for ϕ_H (C-2^f-O-C-H-1^g) and 70° to 90° for ψ_{C-1^f} (C-1^g-O-C-C-1^f). In view of the severe, non-bonded interactions that are present when sucrose is moved from this favored conformation, the *exo*-anomeric effect has no meaningful influence on this conformational preference. However, the inability of the glycosidic bonds of sucrose to adjust to the *exo*-anomeric effect must be expected to result in weaker than normal glycosidic bonds. Consequently, this property must be a contributing factor to the driving force exhibited by so-called, energy-rich sucrose in transglycosylation reactions.

The slight changes in the coupling constants for the vicinal hydrogens of the fructose residue with change in solvent from D_2O to $(CD_3)_2SO$ (8.8→8.1 Hz and 8.6→7.6 Hz for H-3^f and H-4^f, respectively) are in keeping with the flexibility of the furanoid ring. This flexibility is also evident from the fact[10] that changes in temperature caused a greater change for the ^{13}C-chemical shifts of the fructose residue than for the glucose residue. As expected, all of the signals moved to lower field with increasing temperature. For these reasons, we do not consider that sucrose can be considered to be a flexible molecule in the sense of different conformations about the glycosidic bonds being separated by low (<2.5 kcal/mole) differences in energy, except for the very similar conformers expected to reside within the steep energy-wells depicted in Fig. 1. Both the ^{1}H- and ^{13}C-chemical shifts remained essentially the same on changing the concentration of sucrose in D_2O from 0.3 to 3M.

In our preliminary communication[1], the chemical shifts for C-1^f and C-6^f assigned by Pfeffer and co-workers[11] were reassigned. Our assignment was based on the coupling (5 Hz) of a ^{13}C-atom with H-3^f, which required this atom to be C-1^f. This conclusion has now been reached independently by Jones and co-workers[12].

The temperature shifts reported by Bock and co-workers[10], and based on the previous assignment[11], are corrected in Table II. As discussed in detail by Czarniecki and Thornton[13], the stronger the hindrance to rotation about the C–C bond (including intramolecular hydrogen-bonding) for a hydroxymethyl group, the slower the internal motion and the greater should be the effect on the ^{13}C-chemical shift of an increase in temperature. The hydroxymethyl group of a galactopyranoside is now well established[9] to reside extensively in the orientation that has O-6 in *syn*-axial-like relationship to H-4. Since this orientation does not allow intramolecular hydrogen-bonding and there is no other appreciable restriction to freedom of rotation, increasing temperature would be expected to have little influence on chemical shift. Indeed, as seen in Table II, no observable shift was reported[10] in the range 300–360 K. On the other hand, as pointed out by Czarniecki and Thornton[13], the hydroxymethyl group of a glucopyranoside appears to be intramolecularly hydrogen-bonded, since an important change in chemical shift occurs with change in temperature. As seen in Table II,

TABLE II

EFFECTS OF TEMPERATURE[a] ON THE ^{13}C-CHEMICAL SHIFTS OF HYDROXYMETHYL GROUPS IN CARBOHYDRATES DISSOLVED IN D_2O

	Temperature shifts (Hz/K)[a]		
Sucrose	0.6 (C-6^g)	0.8 (C-1^f)	0.1 (C-6^f)
Methyl β-D-glucopyranoside	0.4 (C-6)	—	—
Methyl α-D-galactopyranoside	0.0 (C-6)	—	—

[a]Measured[10] at 62.89 MHz in the range 300–360 K.

TABLE III

RELAXATION DATA FOR SUCROSE

Position	D-*Glucose residue*						D-*Fructose residue*					
	1	*2*	*3*	*4*	*5*	*6*	*1*	*2*	*3*	*4*	*5*	*6*
^{13}C-T_1 values (s)[a]	0.46	0.48	0.51	0.47	0.48	0.29	0.27	—	0.46	0.46	0.44	0.32
^{1}H-T_1 values (s)[b]	0.89	1.15	1.69	1.33	—	—	—	—	1.57	1.34	—	—
C values[c]												
Conformer A	80	77	85	82	—	—	—	—	78	84	—	
Conformer B	80	77	85	82	—	—	—	—	78	90	—	
Conformer C	80	77	85	82	—	—	—	—	78	138		

[a]Measured at 305 K and 67.89 MHz for a 0.5M solution in D_2O, using the inversion–recovery method. [b]Measured at 295 K and 400 MHz for a 0.1M solution in D_2O, using the inversion–recovery method. [c]Calculated as described in ref. 9, using the minimum energy conformation (Fig. 1) which set the glycosidic bonds with the torsion angles $\phi^H = -20°$ and $\psi^{C-1f} = 80°$. The conformers A, B, and C are described in Fig. 2.

the change was reported to be 0.4 Hz/K. On this basis, an important restriction to freedom of rotation is present for C-6^g and C-1^f, but not for C-6^f, of sucrose (Table II). These observations are in complete accord with the ^{13}C-T_1 relaxation rates reported in Table III and lend strong support for the presence of a serious barrier to the freedom of rotation about the C-5^g to C-6^g bond. In the case of C-1^f, the restriction to rotation, as will be seen below, is best assigned to the presence of an intramolecular hydrogen-bond between O-1^f and O-2^g. It is interesting to note, in this regard, that the signals for H-1^f and H-1$'^f$ could not be resolved at 400 MHz in either D_2O or $(CD_3)_2SO$. That this situation exists because of preferred orientation became evident when it was observed that heating of both solutions caused the signals of these protons to appear as an AB pattern. Thus, the evidence[1] seems to be overwhelmingly in favor of the existence of the OH-1^f to O-2^g hydrogen-bond in water as well as in $(CD_3)_2SO$.

From the ^{13}C-T_1 relaxation times presented in Table III, it is evident that the sucrose molecule tumbles isotropically when in aqueous solution. The measured values for the secondary carbons are all within 0.47 $\pm$0.04 s and these results are expected to be accurate to within 0.05 s. In the absence of freedom to rotation about the C–C bonds, the relaxation times of the primary carbon atoms would be expected to be half those of the secondary atoms, *i.e.*, 0.24 s. As seen in Table III, these values are C-1^f = 0.27, C-6^g = 0.29, and C-6^f = 0.32 s. These data are in accord with those published by Allerhand and co-workers[14] and indicate that the internal motion of the C-1^f hydroxymethyl group is only slightly greater than the overall motion of the molecule. The C-6^g hydroxymethyl group has slightly faster internal motion, but C-6^f appears to rotate much more rapidly. These relative rates of internal motion are in accord with the conclusions reached above based on the effect of temperature on conformational equilibria.

The results of hard-sphere calculations[9,15] of the energy barriers to rotation for the three hydroxymethyl groups were in general agreement with these observations. First of all, it became apparent that there is considerable interaction between the C-6^f and C-6^g hydroxymethyl groups when O-6^g is in the orientation which is preferred for simple glucopyranosides[16], namely, that wherein H-5^g and O-6^g define a torsion angle of near $-60°$. The orientation wherein O-4^g and O-6^g are in a *syn*-axial-like relationship is not very amenable to assessment by hard-sphere calculations[15] but is well known[16] to be energetically unfavorable. Therefore, it is to be expected that O-6^g will favor the conformation in which it defines a torsion angle of near 180° with H-5^g, namely, that conformation in which it occurs in crystalline sucrose[5]. There exists greater freedom to the rotation about the C-5^f–C-6^f bond, because only that orientation which occurs in the crystalline state is seriously unfavorable. Indeed, as can be appreciated from Fig. 2, the formation of an H-6^f–O-5^g hydrogen-bond would place O-6^f in a sterically unfavorable orientation, conformer C, which we estimate (HS calculations) to be ~9.0 kcal/mol less favorable than the other staggered orientations. Under this circumstance, HO-6^f is expected to exist in conformers A and B (Fig. 2) and to be solvated by water. These orientations are,

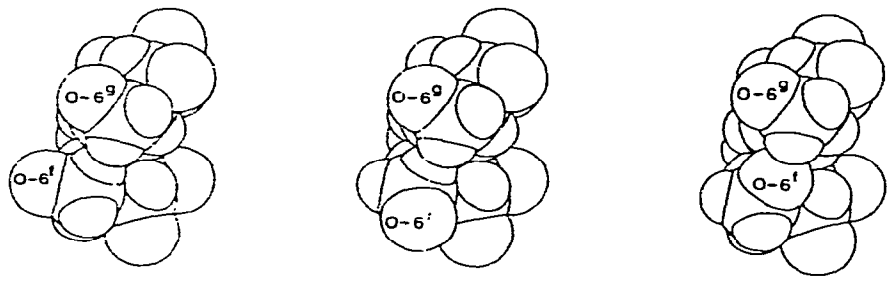


Fig. 2. Computer-drawn CPK models to display the three staggered conformations arising from rotation about the C-5^f–C-6^f bond of sucrose, using ϕ^H and ψ^{C-1^f} values of $-20°$ and $80°$, respectively.

in fact, unequivocally confirmed by the ^{1}H-T_1 values (Table III) for H-4^f. It is seen that the C-values are constant (81 $\pm$4) when these are calculated with the C-6^f hydroxymethyl group in either conformation A or B of Fig. 2, but that a serious discrepancy for H-4^f results when the group is in conformation C. Therefore, HO-6^f is not intramolecularly hydrogen-bonded to O-5^g in the same manner as in the crystalline state. Should HO-6^f be intramolecularly hydrogen-bonded in aqueous solution, then the proton acceptor could be O-5^f which can be separated from O-6^f by 2.85 Å (conformer A, Fig. 2). Evidence will be presented below, in connection with rates of exchange (Table V), where it will be seen that such an intramolecular hydrogen-bond may well be prevalent for sucrose in solution in $(CD_3)_2SO$.

As mentioned above, chemical shift and coupling constants strongly indicated that the sucrose molecule exists in essentially the same conformational equilibrium in solution in $(CD_3)_2SO$ and in D_2O. This contention has now found support in the

TABLE IV

THE SIMILARITY OF THE CONFORMATION OF SUCROSE IN D_2O AND $(CD_3)_2SO$

	Nuclear Overhauser enhancements							
	Resonance for H-1^g saturated Enhanced signal				*Resonance for H-1,1$'^f$ saturated Enhanced signal*			
	H-1,1$'^f$		*H-2^g*		*F-1^g*		*H-3^f*	
	D_2O	*$(CD_3)_2SO$*	*D_2O*	*$(CD_3)_2SO$*	*D_2O*	*$(CD_3)_2SO$*	*D_2O*	*$(CD_3)_2SO$*
Observed[a], %	7.5	6.5	17	15	6.5	9.3	6.5	7.8
Relative	0.31	0.29	0.69	0.71	0.50	0.55	0.50	0.45
Calculated[b], relative	0.34	0.34	0.66	0.66	0.51	0.51	0.49	0.49

[a]Measured in the difference mode. [b]Calculated as described in ref. 9, using the internuclear distances provided by the molecular model described in Fig. 1.

nuclear Overhauser enhancements (n.O.e.) reported in Table IV. It is seen that the four enhancements observed with D_2O as solvent are, well within experimental error, the same as those observed with $(CD_3)_2SO$ as solvent. Also, the agreement between the observed and calculated relative n.O.e. confirm the conformational assignment.

We now wish to comment on exchange reactions between the hydroxyl groups of sucrose solvated by $(CD_3)_2SO$ and low concentrations of water and methanol in this solvent.

Our previous communication[1] reported the chemical shift produced by the introduction of a deuterium atom for hydrogen in a hydrogen-bridged structure involving two hydroxyl groups: O-H←O-H, O-D←O-H, and O-H←O-D. The hydrogens in the half-exchanged structures produced their signals at different fields than did the corresponding hydrogens in the dihydroxy form[1]. A similar situation was to be expected for H_2O and HOD strongly solvated by $(CD_3)_2SO$, and this was observed as follows. The residual water (near 0.03M) present in a sample of pure $(CD_3)_2SO$ provided a signal at 3.696 p.p.m. relative to that (2.480 p.p.m.) for internal acetone (1%). On adding D_2O in a nearly equimolar amount to the H_2O, two signals (one broad signal at 3.694 p.p.m. and a sharp signal at 3.720 p.p.m.) appeared. The deuterium-broadened, high-field signal is assigned to DOH. Evidently the deuterium

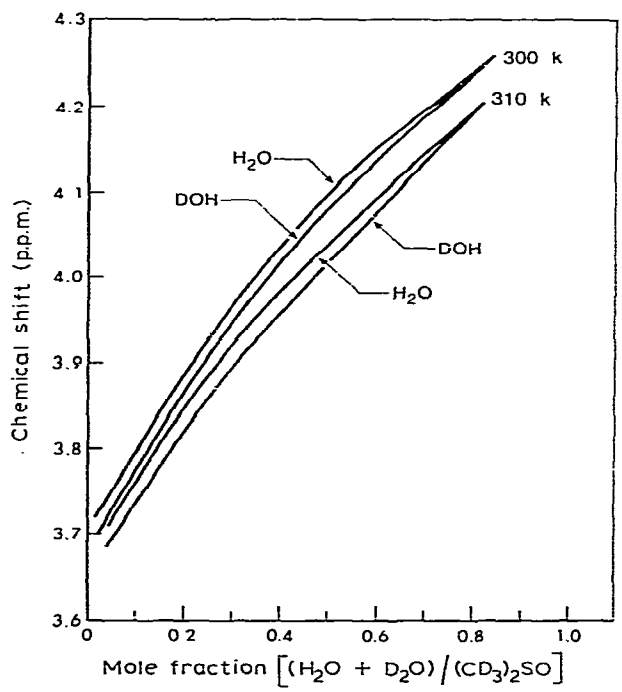
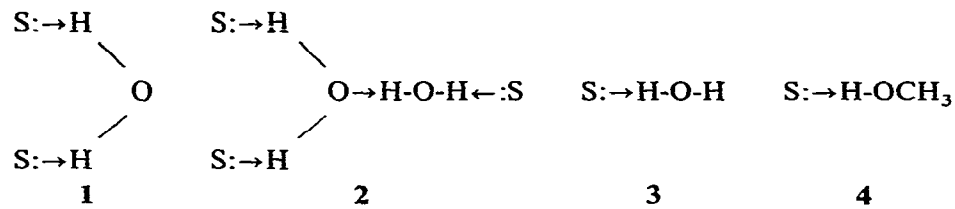


Fig. 3. The change in the chemical shifts for H_2O and DOH in $(CD_3)_2SO$ with increasing content of water.

bond in $(CD_3)_2SO \rightarrow DOH \leftarrow (CD_3)_2SO$ is stronger than the hydrogen bond in $(CD_3)_2SO \rightarrow H\text{-}O\text{-}H \leftarrow (CD_3)_2SO$ and, consequently, the hydrogen atom in the DOH complex is more strongly shielded than are those in the H_2O complex. This result is similar to the stronger shielding that is observed[17] for the ^{13}C atom in the complex $(CD_3)_2SO \rightarrow D\text{-}O\text{-}^{13}C$ as compared to $(CD_3)_2SO \rightarrow H\text{-}O\text{-}^{13}C$. These effects are related to the increased strength of the hydrogen bond between the hydroxyl groups in structures of the type $(CD_3)_2SO \rightarrow H\text{-}O \rightarrow H\text{-}O$, because of the coordination with the

$(CD_3)_2SO$[1,18]. The influence of the greater strength of the deuterium bond is also evident from the shortened $^{13}C\text{-}T_1$ relaxation times[14] for sucrose when dissolved in D_2O as compared to H_2O.

The results obtained on adding successive amounts of a D_2O/H_2O mixture to pure $(CD_3)_2SO$ are presented in Fig. 3. As mentioned above, the $(CD_3)_2SO$ slows the exchange between the DOH and H_2O molecules sufficiently for their signals to be detected separately. As seen in Fig. 3, this condition is maintained until the ratio of the water (H_2O + D_2O) to $(CD_3)_2SO$ molecules approaches 1. The two signals begin to merge when the mole fraction of water is near 0.5. Coalescence is complete when the mole fraction is 1. When the mole fraction of water is 0.5 or less, it is expected that the water molecules exist very extensively in the trimeric complex **1**. As the water content is increased above a mole fraction of 0.5, the tetrameric species **2** is formed. Proton exchange involving water from **2** is also very slow and separate signals for the DOH and H_2O molecules are observed until the mole fraction of the water is above 2/3. At higher mole fractions, the species **3** should become readily available and probably is responsible for the rapid exchange which causes the signals to coalesce as the mole fraction approaches 1. In contrast, in an excess of $(CD_3)_2SO$, methanol must exist essentially entirely in the simple complex **4**.



We have previously published[1] the chemical shifts of the hydroxyl-group protons of sucrose dissolved in $(CD_3)_2SO$. The slight difference between those reported and those listed in Table V are attributed to very slight differences in temperature and water content. As seen in Table V, an increase in temperature caused all of the OH signals to move, as expected[6], to higher field. As pointed out by Casu and co-workers[6], the solvation of carbohydrates by this solvent is a complex phenomenon and the temperature dependencies are not subject to simple interpretation. Nevertheless, it may be noted that the chemical shift of the one hydroxyl group (OH-1^f) that is known[1] to be intramolecularly hydrogen-bonded was least temperature-dependent. Although marginal, this observation concurs with the opinion proposed by St.-

TABLE V

INFLUENCE OF TEMPERATURE AND ADDED SOLVENTS ON THE ^{1}H-CHEMICAL SHIFTS OF THE HYDROXYL PROTONS OF SUCROSE (0.1M) DISSOLVED IN $(CD_3)_2SO$

	D-*Glucose residue*				D-*Fructose residue*			
	OH-2	*OH-3*	*OH-4*	*OH-6*	*OH-1*	*OH-3*	*OH-4*	*OH-6*
Chemical shift (s)[a]	5.361	5.070	5.061	4.713	5.120	4.800	5.502	4.694
Temperature dependence[b] (p.p.m./K × 10^3)	−5.40	−6.13	−4.85	−5.59	−4.60	−5.18	−4.67	−4.84
Effect of added solvent (p.p.m./mL)[a]								
DOH[c]	1.89	1.81	2.91	3.09	2.22	3.05	3.39	3.04
CD_3OD[d]	0.31	0.37	0.25	0.21	0.05	0.21	0.36	0.01

[a]Measured at 270 MHz and 310 K using a 0.1M solution of sucrose, with acetone as the internal reference (2.480 p.p.m.). [b]Determined in the range 300–360 K. [c]Determined by adding up to 50 μL of a 1:1 mixture of D_2O and H_2O to 0.5 mL of the sucrose solution. The slope (p.p.m./mL) was determined from the linear regression of the chemical shifts. [d]As in (*c*), but adding CD_3OD.

Jacques and co-workers[19]. When DOH($D_2O \rightleftharpoons H_2O$) was added to the solution of sucrose in $(CD_3)_2SO$, all of the OH-chemical shifts, of course[6], moved to lower field. One might have expected that OH-1^f would be least influenced, since the change in chemical shift with increased content of water is related to the rate of exchange of the hydroxyl group with water, and OH-1^f is intramolecularly hydrogen-bonded. Indeed, OH-1^f exchanged more slowly than most hydroxyl groups, but not slower than OH-2^g or OH-3^g. We conclude that this also is a highly complex phenomenon which is not subject to simple explanation. However, as seen in Table V, the addition of CD_3OD caused changes in chemical shifts which were remarkably slower for all of the hydroxyl groups, but two, namely, OH-1^f and OH-6^f, exchanged much more slowly than did the others. The very slow change observed for OH-1^f can be assigned to its involvement in the intramolecular hydrogen-bond to OH-2 and confirms[1] that the hydrogen bond is essentially in only one direction; namely, from OH-1^f to O-2^g, as in crystalline sucrose. If this postulate is accepted, the conclusion would be drawn that OH-6^f is intramolecularly hydrogen-bonded (as well as OH-1^f) for sucrose dissolved in $(CD_3)_2SO$. This is acceptable since, in the orientation wherein O-6^f defines a torsion angle of $-60°$ with H-5^f (conformer A of Fig. 2), OH-6^f is in close proximity to both O-5^f and O-5^g. The point of interest is that this intramolecular hydrogen-bond, if it actually exists, is formed from an orientation for the C-6^f hydroxymethyl group which is different from that involved in the formation of the OH-6^f to O-5^g intramolecular bond that is present in crystalline sucrose.

Lemieux[20] has recently reported convincing evidence that the main driving-force for the binding of oligosaccharides to proteins is hydrophobic in nature, involving an interaction between a hydrophobic cleft in the surface of the protein and a hydrophobic portion of the carbohydrate structure. Hydroxyl groups which penetrate the hydrophobic cleft of the protein appear generally to do so in an intramolecularly hydrogen-bonded form[21]. The interaction of sucrose with the receptor site which is concerned with the sweet-taste response can be expected to involve similar phenomena. Indeed, in view of the hydrogen bond that exists between O-1^f and O-2^g, a substantial portion of the α^f,β^g-side of the sucrose molecule is amenable to engagement in hydrophobic bonding. This topography would include parts of the surface described by H-3^f, O-5^f, O-5^g, and the surface involving H-1^f, H-$1'^f$, O-1^f... H...O-2^g, H-1^g, and H-2^g. It is well known that sucrose binds only very weakly with proteins and this would be in keeping with the notion[20] that, for strong binding, the hydrophobic portion of the carbohydrate should be wedge-shaped in order to penetrate satisfactorily the hydrophobic cleft of the receptor site. The above-described hydrophobic region of sugar would only fit a rather shallow cleft and only weak binding is to be expected. This matter is raised because it appears to offer an insight on sweetness. For example, the replacement of OH-1^f of sucrose by chlorine is known to increase the sweetness[22] strongly and this is in keeping with the essentially hydrophobic nature of the interaction with the receptor site. Inversion of C-4^g of sucrose would bring the OH-4 group over the hydrophobic region and, indeed, 4^g-epi-sucrose is reported[23] not to be sweet. An examination of saccharin readily shows a

near isomorphous relationship of this molecule with the hydrophobic region of sucrose. Interestingly, when the structures are superposed, the NH grouping of saccharin occupies a region near that of OH-2^g. It can be postulated, therefore, that the taste response is triggered by the involvement of these active hydrogens with a proton acceptor near the periphery of the receptor site. These observations are considered to offer an attractive working hypothesis for the study of structure–sweetness relationships[24] and are being examined in further detail.

EXPERIMENTAL

The n.m.r. spectra were recorded with a Bruker WH-400 instrument operating in the Fourier-transform mode at 310K unless otherwise specified. ^{1}H-N.m.r. spectra were obtained for 0.1M solutions at 400 MHz, using 90° pulses (12 μs) and a digital resolution of $\pm$0.2 Hz. Acetone (1%) was used as the internal reference (2.480 p.p.m.). Assignment of signals was based on double-resonance experiments. ^{13}C-N.m.r. spectra were obtained for 0.3M solutions at 100.13 MHz, using 90° pulses (25 μs), broadband proton-decoupling, and a digital resolution of $\pm$10 Hz. 1,4-Dioxane (2%) was used as the internal reference (67.40 p.p.m.).

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