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LONG-RANGE CARBON–PROTON COUPLING CONSTANTS: APPLICATION TO CONFORMATIONAL STUDIES OF OLIGOSACCHARIDES

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ABSTRACT

$^3J_{C,H}$ values have been measured using selective 2D heteronuclear *J*-resolved n.m.r. spectroscopy for the COCH fragment in various carbohydrates. Measurements on model compounds have been used to characterise a Karplus-type relationship between $^3J_{COCH}$ and dihedral angles in sugar. The $^3J_{C,H}$ values have also been measured for C-2′–O-2′–C-1–H-1 of sucrose and the sucrose residues in raffinose, stachyose, and melezitose. These values are similar to each other for solutions in D_2O and $(CD_3)_2SO$ and are little affected by the change in solvent, but differ from those predicted from the crystal conformations. The method has been used to correct some assignments in the published ^{13}C-n.m.r. spectrum of melezitose.

INTRODUCTION

The potential value of $^3J_{C,H}$ values in the conformational analysis of carbohydrates is well recognised, but their use has been limited by technical difficulties of measurement. Several studies of ^{13}C-enriched and selectively deuterated compounds[1–5] provided sufficient data for characterisation[6] of a Karplus-type curve[7] relating $^3J_{C,H}$ values to dihedral angles for C–O–C–H of the glycosidic bond in sugars. This curve has been used in conformational studies of disaccharides[8–10].

Several 2D-n.m.r. techniques have been reported[11–13] to be capable of identifying specific long-range carbon–proton couplings without the need for ^{13}C enrichment or selective deuteration. The simplest of these techniques is a heteronuclear *J*-resolved method[13] which can be used to measure accurately, in one

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experiment, all the long-range $J_{C,H}$ values for a selected proton. The method has been applied to mono-, di-, and oligo-saccharides[14–17].

Using this 2D-n.m.r. method, the $^3J_{C,H}$ values have been determined for the C–O–C–H path of several model compounds and some of the data used to characterise the Karplus curve[6] have been re-measured more accurately. The data differ from that of previous work[6], especially for angles around 180°, and a modified Karplus curve is proposed.

The same method has been used to compare the conformational properties of the O-2′–C-1 glycosidic bonds of sucrose and related oligosaccharides in solution with those in the solid state, and to investigate the effect of solvent on the conformational equilibrium. The method has also been used to correct some assignments in the published ^{13}C-n.m.r. spectrum of melezitose[18].

RESULTS AND DISCUSSION

Characterisation of the Karplus-type curve for three-bond carbon–proton coupling constants. — The $^3J_{C,H}$ values used to derive the Karplus curve illustrated in Fig. 1 are listed in Table I. Model compounds were chosen to provide C–O–C–H fragments with well-defined conformations in solution. The glycosidic linkages of cyclomalto-hexaose and -heptaose (α- and β-cyclodextrin) were used as the best available source of data for dihedral angles in the region 0–20° and several C–O–C–H paths in 1,6-anhydro-α-D-glucopyranose were used for angles in the range 100–170°. The $J_{H\text{-}1,C\text{-}5}$ values for β-D-Glc and β-D-Gal residues were measured in order to provide points for dihedral angles around 60°, and the same coupling path for α-D-Glc residues gives data on dihedral angles around 180°. Differences in

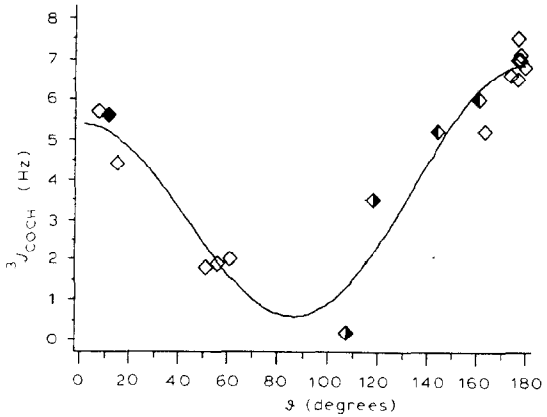

Fig. 1. Relationship between $^3J_{C,H}$ (J) and dihedral angle (θ) for the C–O–C–H fragment of carbohydrates. Variations in substitution: H–C(C–O)–O–C(C–C–H), ◇; H–C(C–C)–O–C(C–O–H), ◆; H–C(C–O)–O–C(C–O–H), ◈; H–C(C–H)–O–(C–O–H), ◈. The curve was calculated as $J = 5.5 \cos^2 \theta - 0.7 \cos \theta + 0.6$ by least-squares fitting of the data in Table I.

TABLE I

TORSIONAL ANGLES AND COUPLING CONSTANTS (J) FOR C–O–C–H PATHWAYS IN SUGARS

	θ COCH (°)[a]	J_{COCH} (Hz)	T (K)
1,6-Anhydro-β-D-glucopyranose			
C-5–O-5–C-1–H-1	163	5.2	303
C-6–O-6–C-1–H-1	144	5.2	303
C-1–O-5–C-5–H-5	161	6.0	303
C-1–O-6–C-6–H-6 endo	118	3.5	303
C-1–O-6–C-6–H6 exo	107	0.2[b]	303
Cyclomaltohexaose			
C-5–O-5–C-1–H-1	177	7.5	323
C-4′–O-4′–C-1–H-1	15	4.4	323
Cyclomaltoheptaose			
C-5–O-5–C-1–H-1	178	7.0	323
C-4′–O-4′–C-1–H-1	7	5.7	323
C-1–O-4′–C-4′–H-4′	11	5.6	323
Cellobiose [*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose]			
C-5–O-5–C-1–H-1 (non-reducing Glc)	56	1.9	323
Maltose [*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose]			
C-5–O-5–C-1–H-1 (non-reducing Glc)	178	7.1	303
Methyl β-D-galactopyranoside			
C-5–O-5–C-1–H-1	51	1.8	303
Methyl β-D-glucopyranoside			
C-5–O-5–C-1–H-1	61	2.0	303
α,α-Trehalose (α-D-glucopyranosyl α-D-glucopyranoside)			
C-5–O-5–C-1–H-1	177	7.0	323
Sucrose (β-D-fructofuranosyl α-D-glucopyranoside)			
C-5–O-5–C-1–H-1 (Glc)	174	6.6	313
Raffinose [*O*-α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl β-D-fructofuranoside]			
C-5–O-5–C-1–H-1 (Glc)	180	6.8	313
C-5–O-5–C-1–H-1 (Gal)	177	6.5	313

[a]Angles taken from crystal structures in the Cambridge Structural Database[19], data from neutron diffraction studies being preferred where available: 1,6-anhydro-β-D-glucopyranose[20,21], cyclomaltohexaose[22,23], cyclomaltoheptaose[24], cellobiose[25,26] methyl β-D-galactopyranoside[27], α,α-trehalose[28], sucrose[29], and raffinose[30]. For maltose, angles were estimated from the crystal structure of α-D-glucopyranose[31], and for methyl β-D-glucopyranoside from the crystal structure of β-D-glucopyranose[26,32]. [b]Estimated from the difference in line-width of the C-1 signal compared with that of the C-6 signal.

substituents for the coupling paths may affect the magnitudes of the coupling constants[33], but this effect is expected to be small compared with that for changes in dihedral angles.

The new data were fitted by least-squares minimisation to a curve of the form $J = A\cos^2\theta + B\cos\theta + C$. The value of 0.2 Hz for the point at 107° is too small to measure directly and was estimated from line-width measurements. The resulting curve (Fig. 1) differs from that proposed by Hamer *et al.*[6] most significantly near 180°, where several coupling constants were too large to be accommodated by their results and the version of the Karplus curve summarised in ref. 10.

The spread of experimental data (Fig. 1) may be due to the use of angles taken from crystal structure data to represent solution structures and the use of coupling constants from model compounds with different patterns of substitution for the C–O–C–H fragment. Nevertheless, the results suggest that, for glycosidic bond angles (ϕ,ψ), prediction of coupling constants from angles may be made to ± 1 Hz and prediction of angles from coupling constants to $\pm 10°$. This level of accuracy is sufficient to allow comparisons to be made with molecular models. Measurement of the relative magnitudes of $^3J_{C,H}$ values can be used also to monitor changes in conformation under changing conditions, as shown by Perez *et al.*[34] for the solvent dependence of the conformation of methyl β-maltoside, and to compare the conformational equilibria of groups of related compounds.

Comparison of the conformations of sucrose and related oligosaccharides in solution and in the solid state. — Raffinose, stachyose, and melezitose each contain the α-D-Glc*p*-(1↔2)-β-D-Fru*f* unit present in sucrose (Fig. 2). Measurement of the $^3J_{H\text{-}1,C\text{-}2'}$ values is straightforward by *J*-resolved 2D-n.m.r. spectroscopy, as both the proton and carbon resonances are well resolved and easily assigned. The values measured for solutions in D_2O and $(CD_3)_2SO$ are listed in Table II together with the coupling constants predicted from the crystal structures of sucrose, raffinose, and stachyose using the curve in Fig. 1. Although, in the solid state, the C-2′–O-2′-C-1–H-1 angle for raffinose is different from that in sucrose and stachyose, for solutions the $^3J_{C,H}$ value for this path is similar for the three oligosaccharides, and for melezitose, for which no crystal structure information is available. For raffinose, the magnitudes of the observed coupling constants for solutions in D_2O and $(CD_3)_2SO$ are greater than predicted, whereas for sucrose and stachyose they are smaller. The discrepancy between the experimental values and those predicted from the crystal structures indicates that sucrose, stachyose, and raffinose do not exist in solution in the conformation found in the solid state.

The similarity of the observed coupling constants in all four oligosaccharides in solution in $(CD_3)_2SO$ suggests that the conformations about the O-2′–C-1 bond are also similar, in agreement with studies of hydrogen-bonding patterns in this solvent[37]. The change in solvent from $(CD_3)_2SO$ to D_2O did not greatly affect the magnitude of this coupling constant, leading to the conclusion that the conformation about the O-2′–C-1 bond is similar in the two solvents, which accords with the results of a study of sucrose[38] except for a small discrepancy between the reported value (4.5 Hz) for $^3J_{H\text{-}1,C\text{-}2}$ and that (3.8 Hz) observed in the present work.

Use of the 2D heteronuclear J-resolved method as an aid in the assignment of ^{13}C resonances. — Literature assignments of appropriate signals for sucrose[39], raffinose[39], and stachyose[36] were confirmed by heteronuclear *J*-resolved measurements. Where an oligosaccharide contains two or more similar residues, there is often a problem in assigning ^{13}C resonances. Batta and Liptak[40] demonstrated the use of $^3J_{C,H}$ values in the assignment of the ^{13}C-n.m.r. spectrum of α-L-Rha-(1→2)-α-L-Rha-(1→4)-α-L-Rha-1-OMe, using a 2D DEPT technique to identify such couplings. The ^{13}C resonances of the two glucose residues of melezitose, α-D-Glc*p*-

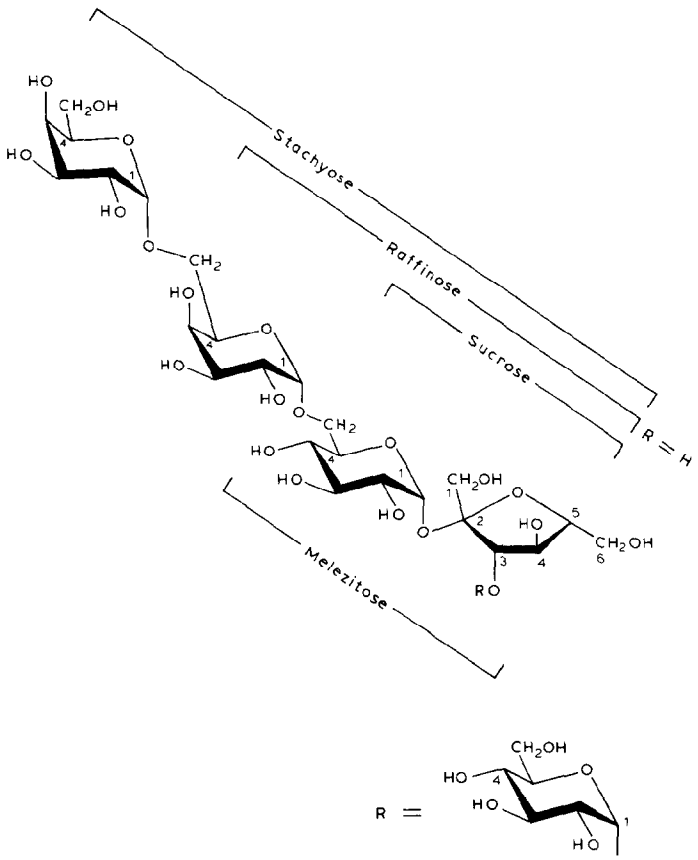

Fig. 2. Structures of sucrose, raffinose, stachyose, and melezitose; adapted from ref. 36.

TABLE II

CARBON-PROTON COUPLING CONSTANTS FOR C-2'–O-2'–C-1–H-1 IN SUCROSE AND RELATED OLIGOSACCHARIDES

Compound	*Crystal structure value for dihedral angle φ (C-2',H-1) (degrees)*	*J predicted from crystal structure*[a] *(Hz)*	$J_{C\text{-}2',H\text{-}1}$ *(Hz)*	
			In D_2O (303 K)	*In $(CD_3)_2SO$ (313 K)*
Sucrose	−8.1[29]	5.3	3.8	4.0
Raffinose	−41.0[30]	3.2	3.8	3.9
Stachyose	−11.2[b]	5.2	3.8	4.0
Melezitose			3.8	4.0

[a]Assuming the solution conformation to be identical to the crystal structure. [b]Calculated from data given in ref. 35.

(1→3)-[α-D-Glc*p*-(1→2)]-β-D-Fru*f*, are difficult to distinguish[36]. The ^{1}H-n.m.r. spectrum of melezitose has been assigned[41]. A 2D heteronuclear *J*-resolved experiment with selective inversion of the resonance (5.45 p.p.m.) of the anomeric proton of the glucose residue attached at C-2′ of fructose gave a spectrum (Fig. 3a) showing coupling to C-2′ of fructose (103.7 p.p.m., 3.8 Hz), and to signals at 73.1 (5.72 Hz, C-3), 72.2 (6.58 Hz, C-5), and 71.0 p.p.m. (1.58 Hz, C-2). The corresponding experiment with inversion of the H-1 resonance (5.18 p.p.m.) of the glucose residue linked at C-3′ of fructose (Fig. 3b) showed coupling to C-3′ of fructose (83.0 p.p.m., 4.06 Hz) and to signals at 72.8 (5.39 Hz, C-3), 72.3 (6.60 Hz, C-5), and 71.4 p.p.m. (1.45 Hz, C-2). The magnitudes of these coupling constants agree with our other measurements on α-D-glucopyranose residues. The C-3 and C-5 signals can be assigned to the correct residue by inspection of the spectra (Fig. 3), thus reversing the assignments of Bock *et al.*[18].

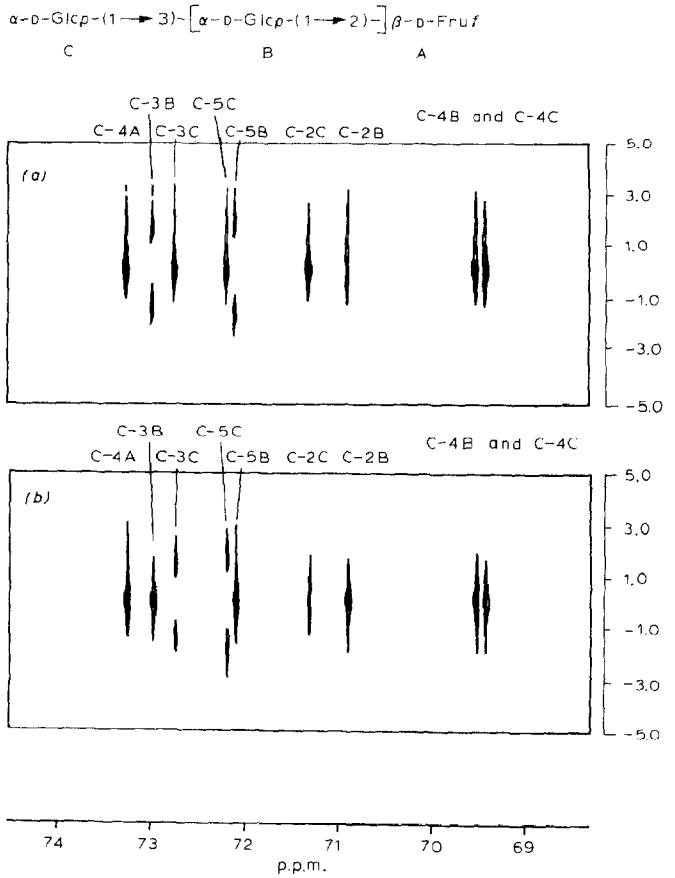

Fig. 3. Partial heteronuclear *J*-resolved 2D spectra for melezitose in D_2O solution. (*a*) Selective inversion of H-1 of α-D-glucopyranose B ($^3J_{C\text{-}2',H\text{-}1}$ = 3.8 Hz). The chemical shift of the signal from C-3B is 73.1 p.p.m. and of C-5B is 72.2 p.p.m. (*b*) Selective inversion of H-1 of α-D-glucopyranose C ($^3J_{C\text{-}3',H\text{-}1}$ = 4.1 Hz). The chemical shift of the resonance from C-3C is 72.8 p.p.m. and of C-5C is 72.3 p.p.m.

EXPERIMENTAL

The 2D heteronuclear *J*-resolved n.m.r. spectra were obtained using the method of Bax and Freeman[13]. Because of instrumental constraints, the 180° selective proton pulse was provided by a DANTE sequence[42], precalibrated by a selective population transfer technique. Experiments were carried out at either 11.75 or 9.4 T, using Bruker AM series spectrometers. Typically, 32 t_1 increments were taken with a 20-Hz spectral width in F_1; in F_2, 4096 data points were acquired over a spectral width of 60 p.p.m. For accurate measurement of coupling constants, the spectra were transformed in F_2 and individual signal-bearing interferograms were then zero-filled and transformed with resolution enhancement to give a final data resolution of 0.05 Hz.pt^{-1}. Assignments of ^{13}C resonances were taken from the literature[18,43]: chemical shifts are quoted relative to that of internal 3-(trimethylsilyl)-1-propanesulphonic acid (−2.7 p.p.m. with respect to external Me_4Si).

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