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Angular dependence of vicinal carbon–proton coupling constants for conformational studies of the hydroxymethyl group in carbohydrates ☆

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Abstract

A theoretical study is presented of the dependence on the hydroxymethyl group conformation of vicinal carbon–proton coupling constant $^3J_{C,H}$ in a series of 16 hexopyranoses. Calculated $^3J_{C,H}$ values for both anomers of D-glucopyranose (**1**), D-mannopyranose (**2**), D-allopyranose (**3**), D-altropyranose (**4**), D-galactopyranose (**5**), D-talopyranose (**6**), D-gulopyranose (**7**), and D-idopyranose (**8**) are based on the FTP formulation in the semi-empirical approximation of INDO. The dependence of the coupling constants on the dihedral angle ω_C between the coupling carbon atom C-4 and protons H-6 is represented by a trigonometric function of the form $^3J_{C,H} = 5.8\cos^2\omega_C - 1.6\cos\omega_C + 0.28\sin 2\omega_C - 0.02\sin\omega_C + 0.52$. It was found that the configuration at the anomeric and C-4 carbon atoms does not show any significant influence on $^3J_{C,H}$ values. Agreement of calculated and experimental values available for mono- and oligo-saccharides is satisfactory. Based on these results, it is concluded that proposed equation for $^3J_{C,H}$ values can be used as a tool for estimation of the conformational properties of the hydroxymethyl group in monosaccharides and of (1 → 6)- linked oligosaccharides in solution.

Keywords: Vicinal carbon–proton coupling constants; Angular dependence; FTP INDO calculations; Hydroxymethyl group conformation

1. Introduction

In many biological processes, the conformations and dynamics of carbohydrates may be of considerable significance. Therefore, to fully appreciate the biological roles of

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carbohydrates, it is necessary to establish the conformations they assume in solution and the ways in which their conformations are affected when protein–carbohydrate interactions occur. The overall conformation of a monosaccharide can be described by the ring shape along with the preferred orientations of exocyclic groups (hydroxyl, hydroxymethyl, *N*- and *O*-acetyl, etc.). For the oligo- and poly-saccharides, the main degrees of conformational freedom in addition to those for monosaccharides, are rotations about the glycosidic torsional angles.

The conformation of the hydroxymethyl group is of interest for interactions involving the hydroxyl group at this position and because the conformation around the C-5–C-6 bond determines the overall shape of oligosaccharides having a $(1 \rightarrow 6)$ glycosidic linkage. The conformational behaviour of the C-5–C-6 linkage in mono- and oligo-saccharides have, therefore, been the subject of several investigations including both experimental and theoretical studies [1].

The exocyclic hydroxymethyl groups in carbohydrates usually exist in three staggered orientations (*gauche–gauche*, GG; *trans–gauche*, TG; and *gauche–trans*, GT; Scheme 1) that correspond to local minima. In both crystal [2] and solution [3], the GT and GG rotamers are preferred for hexopyranoses having a *gluco* configuration, while the TG and GT rotamers are preferred for those having a *galacto* configuration at the C-4 atom. The solution conformation of the hydroxymethyl group is predominantly determined by NMR studies using the three-bond proton–proton coupling constants $^3J_{\text{H-5,H-6}}$ between H-5 and H-6 protons. These coupling constants show a dependence on the angular orientation, denoted as Karplus-type equation [4]. Several parametrization of this relationships were proposed [5,6] and used together with three state model for the interpretation of average values obtained from NMR experiments [1]. Despite their indisputable value in the determination of the hydroxymethyl conformation, quite often unreasonable rotamer distributions having negative population for the TG conformer were calculated using different Karplus-type equations [1].

Over the past decade, it has been shown that similarly to the proton–proton coupling constants, also the carbon–proton coupling constant $^3J_{\text{C,H}}$ are valuable structural probes for determination of conformations of carbohydrates in solution [7,8]. Several equations were proposed to describe an angular dependence on glycosidic torsional angles Φ^{H} and Ψ^{H} of the one-bond and three-bond carbon–proton coupling constants for different C–X–C–H arrays of bonded atoms [9–13]. Three-bond carbon–proton coupling constants between atoms C-4 and H-6 provide a measure of the torsional angle ω around the C-5–C-6 bond and thus can be also used to ascertain the rotamer population about the primary hydroxyl group [14–18]. A prerequisite for the successful application of $^3J_{\text{C,H}}$ values to determine the population of rotamers on C-5–C-6 bond is a reliable angular dependence. The only available relationship for this purpose was determined for compounds having the C-1–C-2R^1(OH)–C-3R^2–H sequence of atoms in which R^1 and R^2 are parts of the alicyclic system. Using experimental results in model compounds, an empirical equation for the angular dependence of $^3J_{\text{C,H}}$ was proposed [19]:

$$^3J_{\text{C,H}} = 5.14\cos^2(\phi - 5.3^\circ) - 0.38\cos(\phi - 5.3^\circ) + 0.52 \qquad (1)$$

where a phase shift of 5.3° was presumed to arise owing to the orientation of the C-2 hydroxyl group relative to the C-3–H bond. However it has long been known, both from

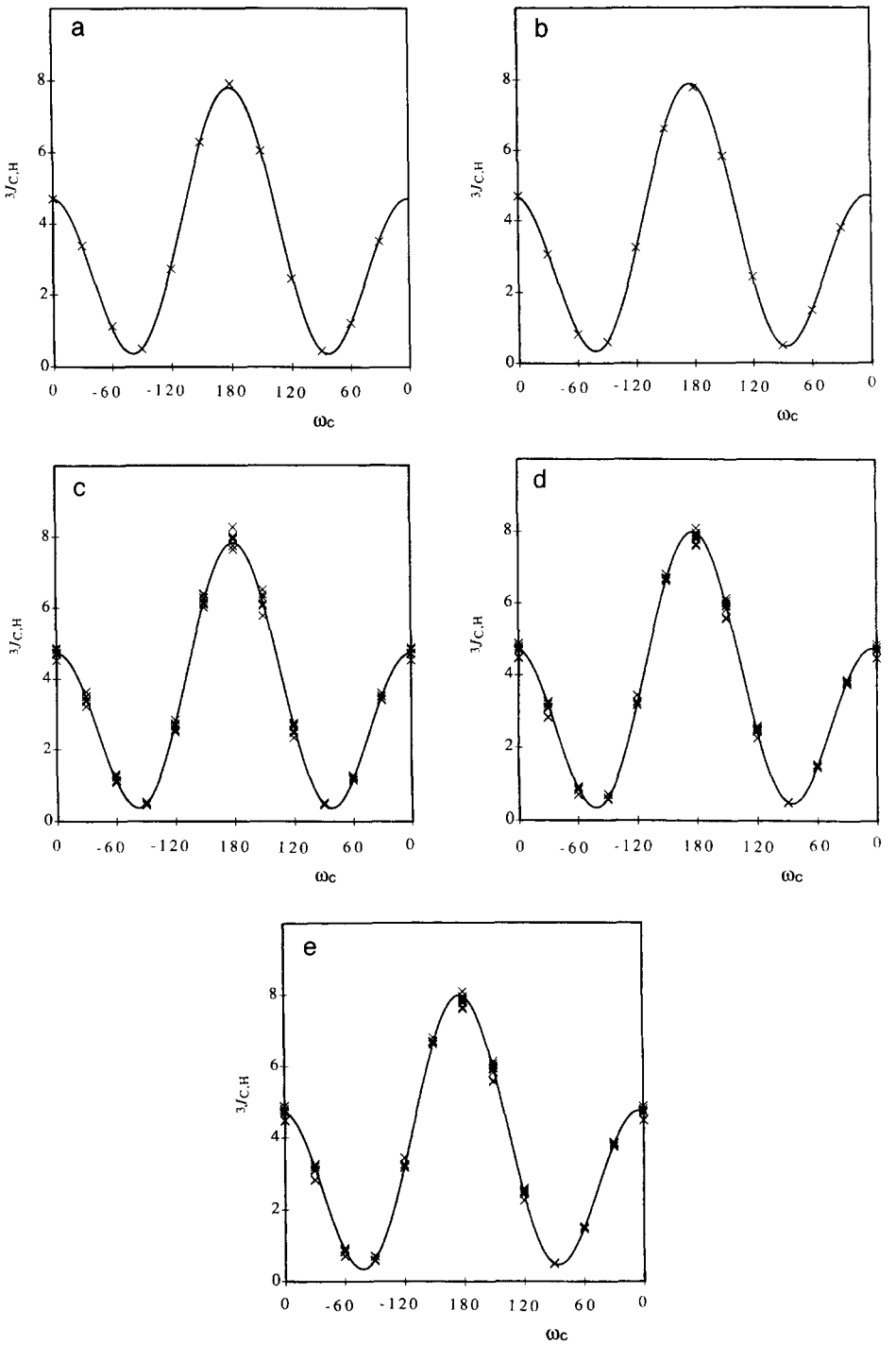
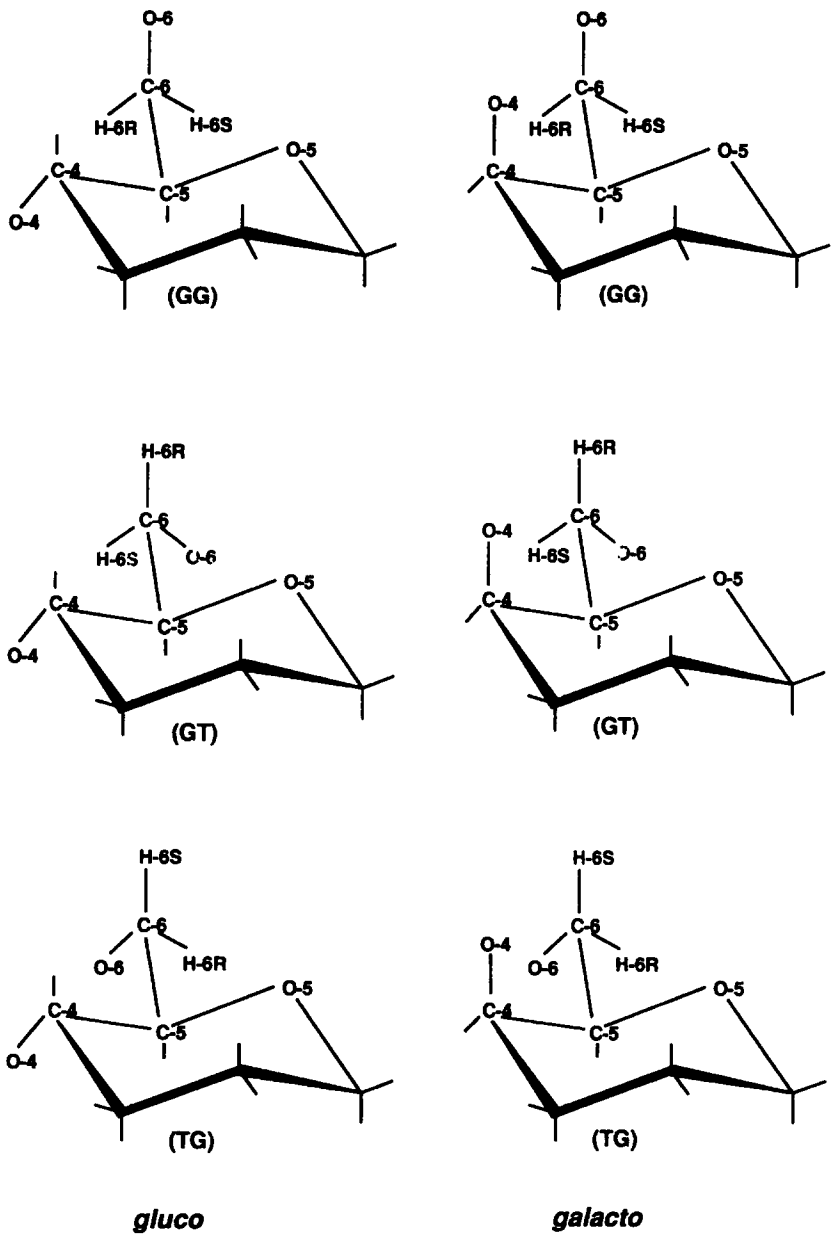


Fig. 1. A plot of the calculated INDO FTP results for the $^3J_{C,H}$ values (Hz) for (a) α-D-glucopyranose; (b) α-D-galactopyranose; (c) hexopyranoses having the *gluco* configuration (**1–4**); (d) hexopyranoses having the *galacto* configuration (**5–8**); and (e) both anomers of all hexopyranoses (**1–8**).

experimental data [20,21] and theoretical calculations [22–27], that $^{3}J_{C,H}$ magnitude is sensitive to several parameters (electronegativity and position of substituents, hybridization, etc.). Since the corresponding array C(OH)–C(O)–C(OH)–H in hexopyranoses has electronegative substituents at the α, β, and γ positions, an application of the latter



Scheme 1. Schematic representation of the three staggered conformations for the hydroxymethyl group of D-hexopyranoses having *gluco* and *galacto* configuration, and the numbering of relevant atoms.

angular dependence is limited. Consequently, more-accurate relationship is needed to allow an improved description of the conformational preferences of the hydroxymethyl group in carbohydrates using the $^3J_{C,H}$ values.

In this study, the dependence of $^3J_{C\text{-}4,H\text{-}6}$ values on the values of ω_C torsional angle has been investigated by quantum chemical method as applied to a series of 16 hexopyranoses. Calculated dependencies were fitted to the relationships in the form of trigonometric equation and the reliability of this dependence is discussed. Finally, the proposed angular dependence was used to interpret available experimental $^3J_{C,H}$ values for mono- and oligo-saccharides.

2. Method

The calculations of $^3J_{C,H}$ were carried out by the finite perturbation theory (FTP) formulation [28] for nuclear spin–spin coupling with the intermediate neglect of differential overlap (INDO) approximation of unrestricted self-consistent-field (SCF) semi-empirical molecular orbitals (MO) theory [29]. The angular dependencies on hydroxymethyl conformations were investigated in a series of 16 hexopyranoses. Geometries of the α- (**a**) and β-anomers (**b**) of D-glucopyranose (**1**), D-mannopyranose (**2**), D-allopyranose (**3**), D-altropyranose (**4**), D-galactopyranose (**5**), D-talopyranose (**6**), D-gulopyranose (**7**), and D-idopyranose (**8**) were based on the standard geometry models for hexopyranoses [30]. The two H-6 protons are magnetically non-equivalent, and in the following the *pro-R* is denoted H-6R and the *pro-S* proton H-6S. The orientation about C-5–C-6 bond is described usually by torsional angle ω (O-5–C-5–C-6–O-6). For the purpose of this investigation, we used torsional angle ω_C (C-4–C-5–C-6–H-6) which corresponds either to the ω_R (C-4–C-5–C-6–H-6R) or to the ω_S (C-4–C-5–C-6–H-6S); and the following relationships hold $\omega \approx \omega_R$ and $\omega_S \approx \omega_R - 120°$. Corresponding three-bond carbon–proton coupling constants will be denoted $^3J_{C,H}$, ^3J_R and ^3J_S, respectively. The schematic representation of three staggered C-5–C-6 rotamers for the hydroxymethyl group in D-hexopyranoses having the *gluco* and *galacto* configuration and the numbering of relevant atoms are shown in Scheme 1.

3. Results and discussion

Molecular orbital description of the conformational dependence of $^3J_{C,H}$ *values.*—The $^3J_{C,H}$ values between the ring carbon atom C-4 and two protons H-6R, and H-6S of the hydroxymethyl group were calculated at interval of 30° for the α- and β-anomers of all hexopyranoses (**1–8**). The calculated $^3J_{C,H}$ values based on the standard geometries of hexopyranoses [30] are plotted as a function of ω_C in Fig. 1. Our calculations show an obvious "Karplus-like" behaviour along ω_C. The dependence can be represented satisfactorily by equation *2*, which is in the form

$$^3J_{C,H} = A\cos^2\omega_C + B\cos\omega_C + C\sin 2\omega_C + D\sin\omega_C + E \qquad (2)$$

where the sin terms reflect the lack of the 180° periodicity due to substituents of different electronegativity on carbon atom C-5. The range of the values of $^3J_{C,H}$ in **1–8**

Table 1
Calculated $A-E$ (Hz) constants of equation 2 for D-hexopyranoses (**1–8**)

Compound	A	B	C	D	E
1a	5.81	−1.56	0.09	−0.02	0.48
1b	5.78	−1.56	0.11	−0.02	0.48
2a	5.71	−1.48	−0.03	0.01	0.47
2b	5.54	−1.52	0.16	−0.04	0.50
3a	5.81	−1.59	−0.07	0.02	0.49
3b	6.02	−1.65	−0.07	0.02	0.50
4a	5.88	−1.54	−0.14	0.04	0.49
4b	5.83	−1.60	0.13	−0.03	0.51
5a	5.78	−1.64	0.42	−0.05	0.55
5b	5.72	−1.59	0.42	−0.05	0.55
6a	5.91	−1.62	0.34	−0.02	0.54
6b	5.81	−1.59	0.37	−0.03	0.54
7a	5.47	−1.63	0.59	−0.10	0.60
7b	5.52	−1.64	0.58	−0.11	0.59
8a	5.85	−1.63	0.36	−0.03	0.53
8b	5.98	−1.67	0.37	−0.03	0.54
gluco (**1–4**)	5.80	−1.55	0.02	−0.01	0.48
galacto (**5–8**)	5.80	−1.62	0.40	−0.05	0.55
overall (**1–8**)	5.80	−1.60	0.28	−0.02	0.52

is 8 Hz with $^3J_{C,H}$ ($\omega_C = 180°$) > $^3J_{C,H}$ (0°) and $^3J_{C,H}$(90°) ~ 0.5 Hz. The calculated results are fitted to the equation 2 and coefficients $A-E$ are listed in Table 1.

A comparison of calculated ^3J_S and ^3J_R values revealed that both coupling constants have the same angular dependence. Inspection of the data in Table 1 indicates that configuration at the anomeric carbon atom has very little effect on the calculated $^3J_{C,H}$ values. This is also the case for the configuration at C-4, a difference between the calculated angular dependence for hexopyranoses having the *gluco* and *galacto* configuration is very small. Indeed, from the $A-E$ constants only the C constant shows a noticeable variation. In D-hexopyranoses having the *gluco* and *galacto* configuration, the torsional angle C-6–C-5–C-4–O-4 describing the orientation of α-substituent with respect to γ-carbon atom is always in *gauche* orientation. Investigation on α-substituted propanes showed [26] that $^3J_{C,H}$ values are substantially larger when the torsional angle between α-substituent and γ-carbon atom is 180° than when this torsional angle is 60°. Thus, a very small influence of the position of an hydroxyl group at C-4 on the $^3J_{C,H}$ values is in agreement with this finding. Based on the similarity of all calculated dependencies, it seems reasonable to establish a general angular dependence of $^3J_{C,H}$ values for all hexopyranoses. The overall dependence of vicinal carbon–proton coupling constant on dihedral angle ω_C was derived by fitting the $A-E$ constants to calculated $^3J_{C,H}$ values for all 16 hexopyranoses and has the form

$$^3J_{C,H} = 5.8\cos^2\omega_C - 1.6\cos\omega_C + 0.28\sin 2\omega_C - 0.02\sin\omega_C + 0.52 \quad (3)$$

The resulting dependence predicts maxima for $^3J_{C,H}$ at 0 and 180° (4.72 and 7.92 Hz), and minima at 85° (0.45 Hz) and −80° (0.34 Hz).

The reliability of the angular dependence.—Before we address the question of reliability of predicted angular dependence of $^3J_{C,H}$ values, it is noteworthy to notice that the comparison of experimental $^3J_{C,H}$ values from solution NMR data with calculated values using equation *3* is affected at least by the following factors: (i) accuracy of $^3J_{C,H}$ values measured from NMR data; (ii) torsional angles determined from X-ray data contain uncertainties; (iii) the crystal and solution structures may differ; and (iv) experimental data represent ensemble average values over local motions. Errors in the measurement of coupling constants are highly dependent on the type of coupling, the type of experiment used to measure the coupling, and signal overlap. The accuracy of measured $^3J_{C,H}$ values in carbohydrates is usually ± 0.5 Hz. Furthermore, it is generally assumed that pyranose has a rigid chair form. This assumption is very limiting and important variations within the ring itself were found [31,32]. For example, the torsional angles in the glucopyranose chair ring are seen to have range of $\sim 20°$ when the results from crystal structures were analysed [31]. Significant ring deformations were also observed during dynamics simulation [32] of simple monosaccharides. For example, a torsional angle C-1–C-2–C-3–C-4 in α-D-glucopyranose adopted values from -35.8 to $-66.7°$; the value of this angle in the crystal structure [33] is $-51.3°$. This suggests that an estimate of the uncertainty in torsional angles is between 10° and 20°.

The compounds used for a comparison of calculated values with experimental data should fulfil several demands. First, the chosen molecules should be sufficiently rigid to ensure that torsional angle ω_C is well determined. It is also desirable that the ω_C angle is the only angle that takes different values, whereas all other geometrical parameters including those defining the orientations of electronegative substituents remain the same. Unfortunately, there are few experimental measurements of $^3J_{C,H}$ values which can be used to check the reliability of the calculated angular dependence as most of the experimental data have been obtained for flexible molecules. The above demands are fulfilled to some extent in some pathway through the ring of methyl D-glycopyranosides and might be used for a comparison of calculated $^3J_{C,H}$ values with experimental data. The measured $^3J_{C,H}$ values for the C-3–C-4–C-5–H-5 pathway [15,18] in compounds **1**, **2b**, and **3b** and in tetra-*O*-acetyl derivatives of **1**, **2**, and **5** are in the interval of 0.9–2.5 ± 0.5 Hz with a mean value of 2.0 Hz. The corresponding torsional angles based on standard structures of hexopyranoses [30] are in the range 55–60°. Taking into account $\pm 10°$ fluctuations of this torsional angle due to the flexibility of pyranose ring, we may assume variations between 45 and 65°. The calculated $^3J_{C,H}$ values 1.0–2.6 Hz for these magnitudes of torsional angle are in reasonable agreement with experimental results. Similarly, for the C-2–C-3–C-4–H-4 pathway in above compounds having the *gluco* configuration, the measured $^3J_{C,H}$ values are < 1 Hz [15,18]. Estimated dihedral angles for this sequence appeared to have range from -65 to $-72°$. When assuming the flexibility of the ring, they can likely adopt values between -55 and $-75°$. Calculated $^3J_{C,H}$ values 0.3–1.2 Hz for these angles are also consistent with experimental data.

In compounds having the *galacto* configuration, the C-2 and H-4 atoms are in *trans* relationship. The calculated $^3J_{C,H}$ values 6.6–7.9 Hz for ω_C torsional angles of 160–180° are ~ 2 Hz larger than measured $^3J_{C,H}$ values [15,18] of 5.1–5.8 ± 0.5 Hz. A rationalisation of this discrepancy follows from structural difference between the C-2–C-3–C-4–H-4 and C-4–C-5–C-6–H-6 arrays of atoms, namely a different substituents

pattern at γ-carbon atoms C-6 and C-4. A comparison of both arrays shows that whereas two hydrogen atoms are linked to the C-6 carbon atom, only one hydrogen atom is linked to the C-4 carbon atom. It has been found from experimental data [34], and theoretical calculations [24–26] on propane derivatives, that the effect of methyl substitution at the γ-position on $^3J_{C,H}$ values is substantial for $\phi = 180°$ but has very little effect for other torsional angles. The $^3J_{C,H}$ values for C-3-substituted propane derivatives are 2–4 Hz less than the value for propane. This decrease has been ascribed to a γ-substituents effect [25] associated with the nonbonded interactions between substituents at α- and γ-carbon atoms. The pattern of substitution at the C-6 and C-4 carbon atoms is similar to that noted for propane derivatives, i.e. a decrease of $^3J_{C,H}$ values about 2 Hz in $^3J_{C,H}$ (180°) but very little change for other torsional angle.

Some of the other interesting features of the calculated results should be mentioned. A nonsymmetrical character of the experimental $^3J_{C,H}$ values, i.e. lower magnitude of $^3J_{C,H}$ values for torsional angles in *−gauche* orientation in comparison with *+gauche* orientation is adequately predicted by calculated $^3J_{C,H}$ values. This asymmetry is not described by equation *1*. The ~2 Hz difference between the $^3J_{C,H}$ values for $\omega_C = 180°$ calculated by equations *1* and *3* (6.04 versus 7.92 Hz) reflects the importance of the electron-withdrawing substituent (carbon versus oxygen). A comparison of *A–E* constants in equation *3* with that in angular dependence of $^3J_{C,H}$ values based on the experimental results in model compounds having $C^*H_3–C(R^1)OH–C(R^2)–H^*$ fragment [19] (equation *1*) revealed that in both dependencies, the scaling constant is 0.52 Hz. This implies that the lowest magnitude of $^3J_{C,H}$ is likely ~0.5 Hz and indicates that experimental $^3J_{C,H}$ values should range from 0.5 Hz to 7.9 Hz.

Conformational preferences of the hydroxymethyl group.—From the above comparison, it is reasonable to conclude that the angular dependence of $^3J_{C,H}$ values on rotation about the C-5–C-6 bond is consistent with the available experimental results and can be applied for estimating the rotamer populations x_{GG}, x_{GT}, and x_{TG}. These can be calculated on the basis of the equations *4–6*

$$x_{GG} + x_{GT} + x_{TG} = 1 \quad (4)$$

$$^3J_R(GG)x_{GG} + {}^3J_R(GT)x_{GT} + {}^3J_R(TG)x_{TG} = {}^3J_R(\text{exp.}) \quad (5)$$

$$^3J_S(GG)x_{GG} + {}^3J_S(GT)x_{GT} + {}^3J_S(TG)x_{TG} = {}^3J_S(\text{exp.}) \quad (6)$$

where ^3J_R and ^3J_S are the calculated values of the vicinal coupling constants for the H-6R and H-6S protons in the individual GG, GT, and TG rotamers and ^3J_R(exp.) and ^3J_S(exp.) are the corresponding experimental $^3J_{C,H}$ values. The equations can be solved mathematically or graphically. In the graphical approach [35], the limiting values for the three staggered conformations are plotted in a two-dimensional graph with ^3J_R and ^3J_S on two axis. This approach shows that all experimental values except one for methyl β-lactoside, fall within the triangle formed by three limiting values and indicates that equation (3) is reasonably accurate. However, it is evident that estimated distribution of conformers depends on the accuracy of measured $^3J_{C,H}$ (±0.5 Hz) and the estimation of torsion angle (±10°). These accuracies can lead to the uncertainty of 5–10% in the population of conformers.

Table 2
Vicinal $^3J_{C,H}$ coupling constants (Hz) for ideally staggered rotamers about the C-5–C-6 bond of the hydroxymethyl groups in D-hexopyranosides calculated using the equation *3*

	GG	GT	TG
ω	−60.0	60.0	180.0
ω_R	−60.0	60.0	180.0
^3J_R	0.94	1.40	7.92
ω_S	180.0	−60.0	60.0
^3J_S	7.92	0.94	1.40

Rotamer distributions were calculated by using two sets of equations for comparison. In the first treatment, the $^3J_{C,H}$ values were calculated for ideally staggered conformation, i.e. $\omega_C = \pm 60$ and 180°, and are listed in Table 2. In the second, which accounts for differences in orientation of rotamers caused by different arrangements of substituents, values of torsional angles ω_C are based on rotamer structures of methyl D-glycoside models calculated by ab initio molecular orbital method using the 6-31G* basis set [36]. The calculated $^3J_{C,H}$ values for D-hexopyranoses having the *gluco* and *galacto* configurations are given in Table 3. It can be seen that more realistic values of torsional angles for the staggered rotamers result in different $^3J_{C,H}$ values for derivatives having the *gluco* and *galacto* configurations.

Both treatments of the available experimental values on glycosides predict similar general distribution of rotamers but yield different absolute percentages (Table 4). It is noteworthy that whereas the first treatment gave physically unreal solution with negative population of TG rotamer in three cases, the second one only for glucopyranose residue of the methyl β-lactoside. In this case, however, $^3J_R = 0.5$ Hz is very small. This value corresponds to a single conformer having $\omega_C = \pm 40°$. Other possible explanation lies in the accuracy of measured $^3J_{C,H}$ value. For example, an increase of this value by a small amount of 0.3 Hz to 0.8 Hz changes the rotamer distribution to a physically real one, namely GG:GT:TG = 15:84:1. The same change in ^3J_S from 2.8 Hz to 3.3 Hz, does not change the population of TG rotamer and gives unreal distribution 20:83:−3. This

Table 3
Vicinal $^3J_{C,H}$ coupling constants (Hz) for staggered rotamers [a] about the C-5–C-6 bond of the hydroxymethyl groups in D-hexopyranosides having *gluco* and *galacto* configuration calculated using the equation *3*

	ω_R	^3J_R	ω_S	^3J_S
		Gluco		
GG	−64.0	0.73	178.3	7.90
GT	71.8	0.73	−46.6	1.89
TG	179.8	7.92	62.0	1.26
		Galacto		
GG	−65.5	0.66	177.0	7.87
GT	75.2	0.61	−43.4	2.15
TG	−168.7	7.77	73.6	0.66

[a] The magnitudes of torsional angles are based on structures of methyl D-glycoside models calculated by ab initio method using the 6-31G* basis set [35].

Table 4
Vicinal $^3J_{C,H}$ coupling constants for C-4–C-5–C-6–H-6 arrays of bonded atoms measured [14–18] from spectra in several glycosides and calculated populations of the rotamers for the hydroxymethyl group

Compound	^3J_R	^3J_S	Ref.	Rotamer populations [a]			Rotamer populations [b]		
				GG	GT	TG	GG	GT	TG
Methyl β-D-glucopyranoside	1.1	2.3	14 [d]	19.7	83.5	−3.2	7.4	87.5	5.1
Methyl tetra-*O*-acetyl-α-D-									
glucopyranoside	1.5	3.5	15 [c]	36.4	59.5	4.1	28.0	61.3	10.7
mannopyranoside	1.4	3.3	15 [c]	33.7	64.0	2.3	24.5	66.2	9.3
galactopyranoside	1.5	4.5	15 [c]	50.7	44.2	5.1	44.2	43.6	12.1
Methyl tetra-*O*-acetyl-β-D-									
glucopyranoside	1.3	3.3	15 [c]	33.8	65.4	0.8	24.3	67.7	8.0
mannopyranoside	1.2	3.2	15 [c]	32.4	68.3	−0.7	22.5	71.0	6.5
galactopyranoside	1.6	4.7	15 [c]	53.4	39.7	6.9	48.1	38.4	13.5
Methyl β-lactoside									
β-D-Glc	0.5	2.8	14 [d]	27.4	84.4	−11.8	14.8	88.4	−3.2
α-NeuAc-(2 → 6)-β-D-Gal-									
(1 → 4)-β-D-GlcNAc									
β-D-GlcNAc	2.0	5.2	16 [e]	60.2	26.4	13.4	56.9	25.4	17.7
β-D-Gal	1.8	2.9	16 [e]	27.5	64.4	8.1	17.4	66.1	16.5
β-Gentiobiose	1.0	3.0	17 [e]	29.8	74.3	−4.1	18.9	77.3	3.8

[a] Based on torsional angles and $^3J_{C,H}$ values of rotamers shown in Table 2. [b] Based on the torsional angles from ab initio calculations and $^3J_{C,H}$ values shown in Table 3. [c] In C_6D_6. [d] In D_2O. [e] In $D_2O/(CD_3)_2CO$, 9:1.

is consistent with the qualitative analysis based on the graphical analysis which indicates that the population of the TG rotamer depends mainly on the magnitude of ^3J_R, whereas ^3J_S influences the distribution between the GG and GT conformers.

The calculated distributions of rotamers agree well with experimental data, but the populations of the GG rotamer in tetra-*O*-acetyl derivatives [15] are consistently lower than those predicted for parent hexopyranoses on the basis of $^3J_{H\text{-}5,H\text{-}6}$ values. There are however, at least four factors which can contribute to this difference. In principle, the presence of acetate group could affect the magnitude of $^3J_{C,H}$ constants, the localisation and the stability of the staggered conformers. Since the $^3J_{C,H}$ values for tetra-*O*-acetyl derivatives have been measured in the nonpolar deuterated benzene, solvent effects could also influence rotamer distributions. Recent investigation [32] using NMR and dynamics simulation on simple monosaccharides showed that the GT rotamer is the lowest energy conformer of the hydroxymethyl group. Our results agree well with this finding. Rotamer distribution is sensitive also to the configuration of the hydroxyl group at C-4. Proportion of the GT rotamer x_{GT} is larger in compounds having the *galacto* configuration than in *gluco*, while population of the TG rotamer x_{TG} is always smaller. The calculated distribution of C-5–C-6 rotamers in the disaccharide β-gentiobiose GG:GT:TG = 19:77:4 is in good agreement with the estimation [17] from maximum-entropy probability distribution GG:GT:TG = 34:64:0, whereas the application of $^3J_{H\text{-}5,H\text{-}6}$ values gave [17] an inconsistent distribution of GG:GT:TG = 56:57:−13. Similarly, for Gal and GlcNAc residues in the trisaccharide, α-NeuAc-(2 → 6)-β-D-Gal-(1 → 4)-β-D-GlcNAc, estimates [16] of rotamer distribution based on the $^3J_{H\text{-}5,H\text{-}6}$ values (GG:GT:TG

= 19:71:10 for Gal, and GG:GT:TG = 70:30:0 for GlcNAc) are consistent with our results GG:GT:TG = 17:66:17 and GG:GT:TG = 77:19:4, respectively.

4. Conclusions

The vicinal carbon–proton coupling constant $^3J_{C,H}$ were calculated as a function of the hydroxymethyl group conformation using the FTP formulation in the semi-empirical approximation of INDO for both anomers of D-glucopyranose (**1**), D-mannopyranose (**2**), D-allopyranose (**3**), D-altropyranose (**4**), D-galactopyranose (**5**), D-talopyranose (**6**), D-gulopyranose (**7**), and D-idopyranose (**8**) and used to establish Karplus-type dependence of $^3J_{C,H}$ values.

The dependence of the coupling constants on the dihedral angle ω_C between the coupling carbon atom C-4 and protons H-6 is represented in the form

$$^3J_{C,H} = 5.8\cos^2\omega_C - 1.6\cos\omega_C + 0.28\sin 2\omega_C - 0.02\sin\omega_C + 0.52.$$

It was found that the configuration at the anomeric and C-4 carbon atoms does not show any significant influence on $^3J_{C,H}$ values.

Agreement of calculated and experimental values available for mono- and oligo-saccharides is satisfactory. Based on these results, it is concluded that angular dependence of $^3J_{C,H}$ values on ω_C torsional angle presented here might be particularly useful as complementary probe to other types of NMR parameters for studying the C-5–C-6 conformations in carbohydrates.

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