One-bond carbon–proton coupling constants: Angular dependence in β-linked oligosaccharides

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SUMMARY

The angular dependence of $^1J_{C,H}$ in model compounds related to β-linked oligosaccharides has been established by FPT INDO quantum chemical calculations. Values calculated for models of (1→1)-, (1→2)-, (1→3)- and (1→4)-linked disaccharides were compared, and the effect of the orientation of HO-2 elucidated. The angular dependence of $^1J_{C,H}$ on the torsional angles Φ^H and Ψ^H and the solvent dielectric constant (ε) was characterized in the form: $^1J_{C,H} = A \cos2\varphi + B \cos\varphi + C \sin2\varphi + D \sin\varphi + E + F\varepsilon$. The $^1J_{C,H}$ values, measured by DEPT methods for C-1–H-1 and C-X′–H-X′ in cellobiose, cyclic trisaccharide and hexopyranoses were used to adjust the calculated angular dependences. Based on the occurrence of the conformers for agarobiose, neoagarobiose, mannobiose and methyl β-xylobioside, the thermodynamically averaged $<^1J_{C,H}>$ values were calculated. The results obtained ($<^1J_{C-1,H-1}>$ 162.4, $<^1J_{C-4′,H-4′}>$ 147.6 Hz for methyl β-xylobioside; $<^1J_{C-1,H-1}>$ 162.4 and $<^1J_{C-4′,H-4′}>$ 147.6 Hz for mannobiose; $<^1J_{C-1,H-1}>$ 162.8 Hz for neoagarobiose and $<^1J_{C-1,H-1}>$ 163.2 Hz for agarobiose) agree well with the experimental values of 162.7, 147.5, 160.4, 147.2, 160.9 and 165.7 Hz, respectively.

INTRODUCTION

Recently, the potentials of one-bond carbon–proton coupling constants for the investigation of carbohydrate conformation in solution have been reconsidered (Davies et al., 1984, 1985; Tvaroska, 1990; Hricovini and Tvaroska, 1990; Tvaroska and Taravel, 1991). Attention has been focused on the angular dependence of $^1J_{C,H}$ values that involve the anomeric or the aglycon carbon, on the glycosidic torsional angles Φ^H and Ψ^H. These studies showed that the values of $^1J_{C-1,H-1}$ and $^1J_{C-X′,H-X′}$ calculated by means of the finite perturbation theory (FPT) formulation for nuclear spin–spin coupling in the INDO (intermediate neglect of differential overlap) approximation of

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the semi-empirical molecular orbital (MO) theory (Pople et al., 1968) vary characteristically with the torsional angles about glycosidic C–O bonds.

Based on the calculated angular dependence of $^1J_{C,H}$ values in a series of model compounds related to α-linked oligosaccharides in conjunction with experimentally observed values in various rigid carbohydrate derivatives, we have established (Tvaroska and Taravel, 1991) the angular and solvent dependences of $^1J_{C,H}$ values in the form:

$$^1J_{C-1,H-1} = 1.32 \cos 2\Phi^H - 3.38 \cos \Phi^H - 1.05 \sin 2\Phi^H + 1.27 \sin \Phi^H + 168.9 + 0.039\,\varepsilon$$

$$^1J_{C-X',H-X'} = 1.02 \cos 2\Psi^H - 1.81 \cos \Psi^H - 0.19 \sin 2\Psi^H + 0.41 \sin \Psi^H + 145.4 + 0.0294\,\varepsilon$$

where ε is the dielectric constant of the solvent, and Φ^H and Ψ^H are the torsional angles describing the relative orientation of monosaccharide residues (H-1–C-1–O-1–C-X') and (C-1–O-1–C-X'–H-X'), respectively.

In the present study, theoretical and experimental results are used to establish the angular dependence of $^1J_{C,H}$ values in β-linked oligosaccharides.

EXPERIMENTAL

General

The dependence of $^1J_{C,H}$ on Φ^H and Ψ^H studied for 2-methoxy-5-(1e), 4-(2e), 3-(3e) and 2-(tetrahydropyran-2-yloxy) tetrahydropyran (4e), which represent (1→4)-, (1→3)-, (1→2)-, and (1→1)-β-linked disaccharides, respectively, and 5e and 6e which are 3-hydroxy derivatives of 1e related to (1→4)-β-linked disaccharides with the D- and L-*glycero* configuration at C-2. The geometries of 1e – 6e were based on the ab initio optimized geometry of 2-methoxy-tetrahydropyran (Tvaroska and Carver, unpublished data). The value of 115° was used for the C–O–C glycosidic angle. An exception was the investigation of the effect of the C–O–C bond angle on $^1J_{C,H}$ where

1e

4e

2e

5e

3e

6e
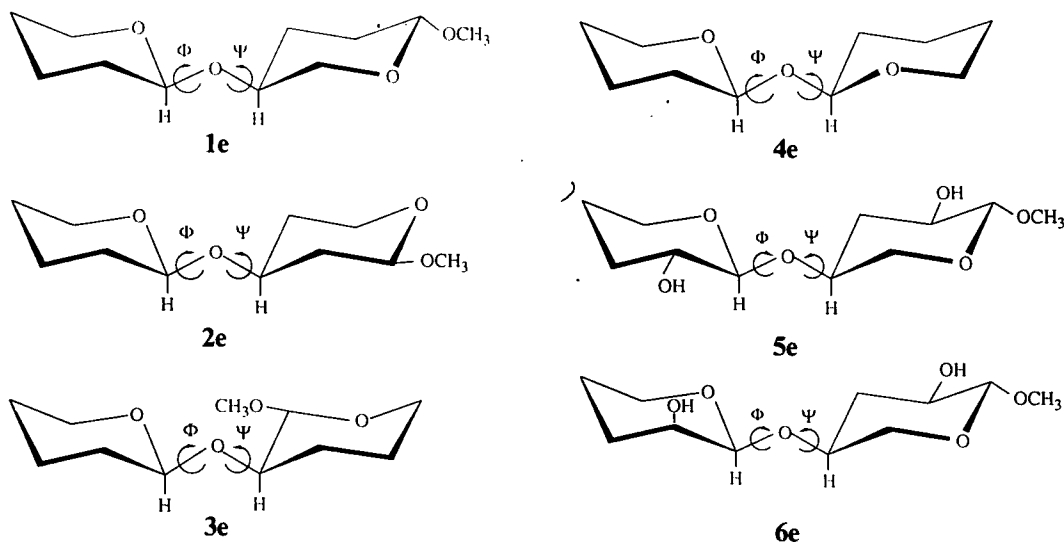
values of 112° and 118° were also used. The $^1J_{C,H}$ values were calculated by FPT-INDO-MO method (Pople et al., 1968).

The following known compounds were chosen to provide H–C–O–C–H fragments with well defined conformations in solution: commercial D-cellobiose (7) (Tvaroska, 1984) from Sigma, D-mannobiose (8) (Tvaroska et al., 1987) prepared in this laboratory from enzymic hydrolysis of a sample of locust bean D-galacto-D-mannan.

Compounds were dissolved (4%) in D_2O. Coupling constants were determined from spectra measured at 30°C with a Bruker AM 400 spectrometer equipped with an Aspect 3000 computer and process controller. DEPT experiments with polarisation transfer from ^1H to ^{13}C nuclei for re-focussed ^1H-coupled spectra were used. The values given are ± 0.5 Hz.

Some $^1J_{C,H}$ were also taken from the literature. This is the case of the cyclic trisaccharide (Bonas
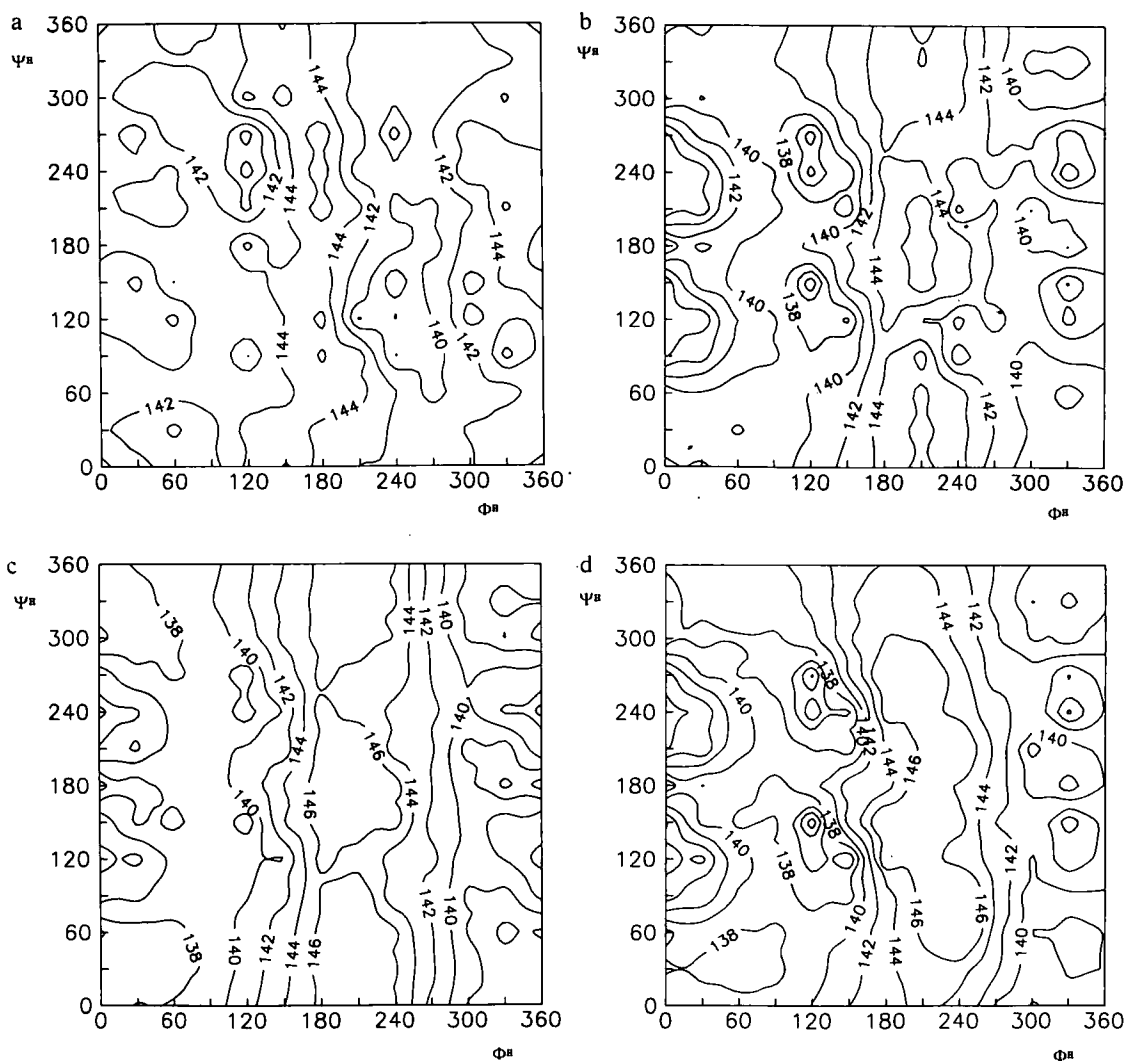

Fig. 1. Contours of the calculated FPT-INDO results for the $^1J_{C-1,H-1}$ values (Hz) for the (1→4)-β-linked oligosaccharide models as a function of Φ^H and Ψ^H for: (a) 1e (τ = 112°), (b) 1e (τ = 115°), (c) 1e (τ = 118°) and (d) 6e.

et al., 1991): [O-β-D-glucopyranosyl(1→6)]₃ 1,6″-anhydride nonacetate (**9**) and methyl β-xyl-obioside (**10**) (Hricovini and Tvaroska, 1990) and of the values observed for the basic trisaccharide unit of agarose (Rochas, personal communication).

RESULTS AND DISCUSSION

MO description of the dependence of $^1J_{C,H}$ on conformation involving the anomeric center

The calculated INDO-FPT $^1J_{C-1,H-1}$ values are expressed as two-dimensional (Φ^H, Ψ^H) maps. The (Φ^H, Ψ^H) maps were prepared by rotating the rigid residue to each combination of Φ^H and Ψ^H on a 30° grid and evaluating the $^1J_{C,H}$ value.

The calculated results for (1→4)-linked molecules are plotted in Fig. 1. The map calculated for **5e** was similar to that for **1e** ($\tau = 115°$) and is not shown. The calculated values were in the range of 130–150 Hz. The minimum values were found for Φ^H 0°, 90° and 300°. The maximum values corresponded to orientations between 180°–240°. An exception is the map for **1e** ($\tau = 112°$) where the minima were shifted to Φ^H 60° and 270° and the maximum was situated at 150°. The $^1J_{C-1,H-1}$ values for Φ^H between 120° and 300° imply that they do not depend on the value of Ψ^H. Because of steric interactions between C-1–H-1 bond and atoms of the aglycon, the $^1J_{C-1,H-1}$ values for Φ^H 0° depend on Ψ^H, and the extreme values were observed for Ψ^H 120° and 240°. The $^1J_{C,H}$ values from these conformers were discarded during further study. Comparison of the map for **1e** (Fig. 1a,b,c) shows how the enlargement of the bond angle τ reveals steric interactions which, as a consequence, decrease the dependence of the $^1J_{C,H}$ values on Ψ^H. The influence of orientation of the 2-substituent in β-anomers on $^1J_{C-1,H-1}$ values is small, and the main features of maps for **1e**, **5e** and **6e** are similar.

Similar to the case of α-linked oligosaccharide models (Tvaroska and Taravel, 1991), the variation of $^1J_{C,H}$ is one of the consequences of the anomeric and exo-anomeric effects. Since the orientation of the lone pairs on the ring oxygen with respect to the C-1–H-1 bond remains fixed, the
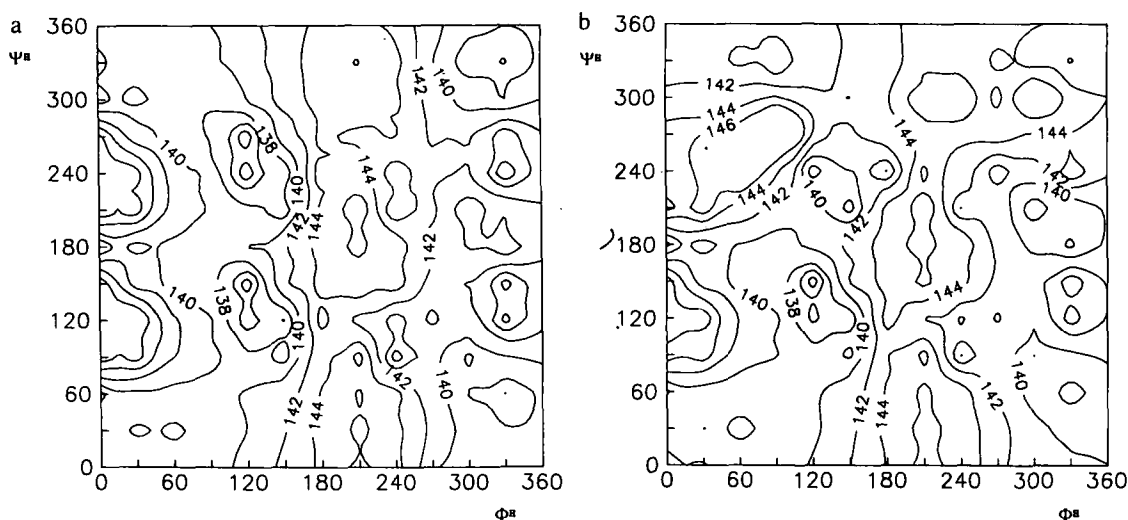

Fig. 2. Contours of the calculated FPT-INDO results for the $^1J_{C-1,H-1}$ values (Hz) of: (a) the (1→3)-β-linked molecule **2e**, and (b) the (1→2)-β-linked molecule **3e**.
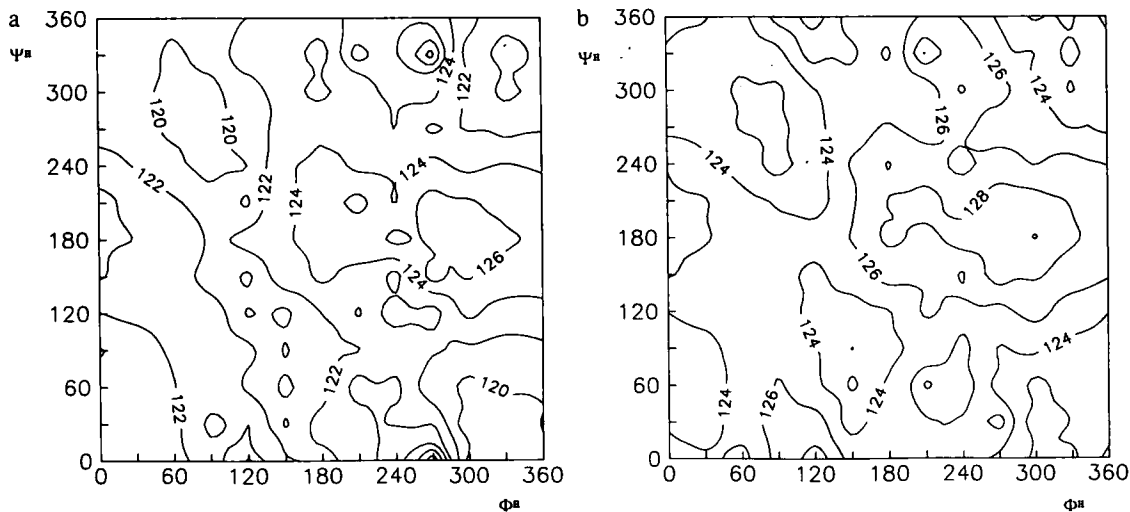
Fig. 3. Contours of the calculated FPT-INDO results for the $^1J_{C-X',H-X'}$ values (Hz) of: (a) the (1→4)-β-linked molecule **1e**, and (b) the (1→3)-β-linked molecule **2e**.

main factor determining the conformational dependence of $^1J_{C-1,H-1}$ is the rotation about the C-1–O-1 bond which changes the orientation of the lone pairs on O-1.

Fig. 2 shows how the type of the glycosidic linkage influences $^1J_{C-1,H-1}$ values. Comparison of the contour map calculated for (1→3)-linked compound **2e** (Fig. 2a) with that for **1e** (Fig. 1b) revealed the main features to be similar. The shape of the map for **3e** is influenced by steric interactions of MeO-1 and the C-1–H-1 bond in a domain with Ψ^H 210°–360°. The domains where these interactions do not occur are similar to those for **1e**.

The aglycon C–H bond

In contrast to α-linked oligosaccharide models, where strong interactions between the C–H bond and ring atoms prevent the calculation of $^1J_{C-X',H-X'}$ values for values of Φ^H between 90° and 270°, in β-linked models such a situation does not occur. Therefore the FPT-INDO method yields $^1J_{C,H}$ values for the whole range of Φ^H and Ψ^H angles. The calculated $^1J_{C-X',H-X'}$ values of **1e–6e** are in the interval of 116–128 Hz and ~20 Hz lower than those for $^1J_{C-1,H-1}$. The conformational dependence of $^1J_{C-X',H-X'}$ are shown in Fig. 3 where the contour of $^1J_{C-X',H-X'}$ for the (1→4)-β-linked molecule **1e** and the (1→3)-β-linked molecule **2e** are plotted. Similar to α-linked models, the conformational dependence of $^1J_{C-X',H-X'}$ is comparable in all studied molecules. The variation of $^1J_{C-X',H-X'}$ for an individual molecule is lower than 6 Hz and the magnitude increases from the C-2'–H-2' bond to the C-4'–H-4' and C-3'–H-3' bonds.

The angular dependence of $^1J_{C,H}$

It has been shown (Hricovini and Tvaroska, 1990; Tvaroska and Taravel, 1991) that $^1J_{C,H}$ values in carbohydrates follow a dependence on the torsional H–C–O–C angle in the form of Eq. 1:

$$^1J_{C,H} = A\cos 2\varphi + B\cos\varphi + C\sin 2\varphi + D\sin\varphi + E \qquad (1)$$

Therefore, this equation was used to establish the angular dependences of the calculated $^1J_{C,H}$ values for β-linked saccharides. Because of steric interactions, several calculated $^1J_{C,H}$ values were discarded and the number of values used to derive constants A–E in Eq. 1 varied from 45–140. The data were fitted by the program COMPLEX (Box, 1965). The resulting curves for the selected compounds are shown on Figs. 4 and 5 and the constants are listed in Tables 1 and 2. The mean values for (1→4)-linked molecules and all molecules except the (1→1)-linked model (4e) are also included. Since the magnitude of the C–O–C glycosidic bond angle is frequently > 115°, the data where τ is 118° were considered in calculating the mean values.

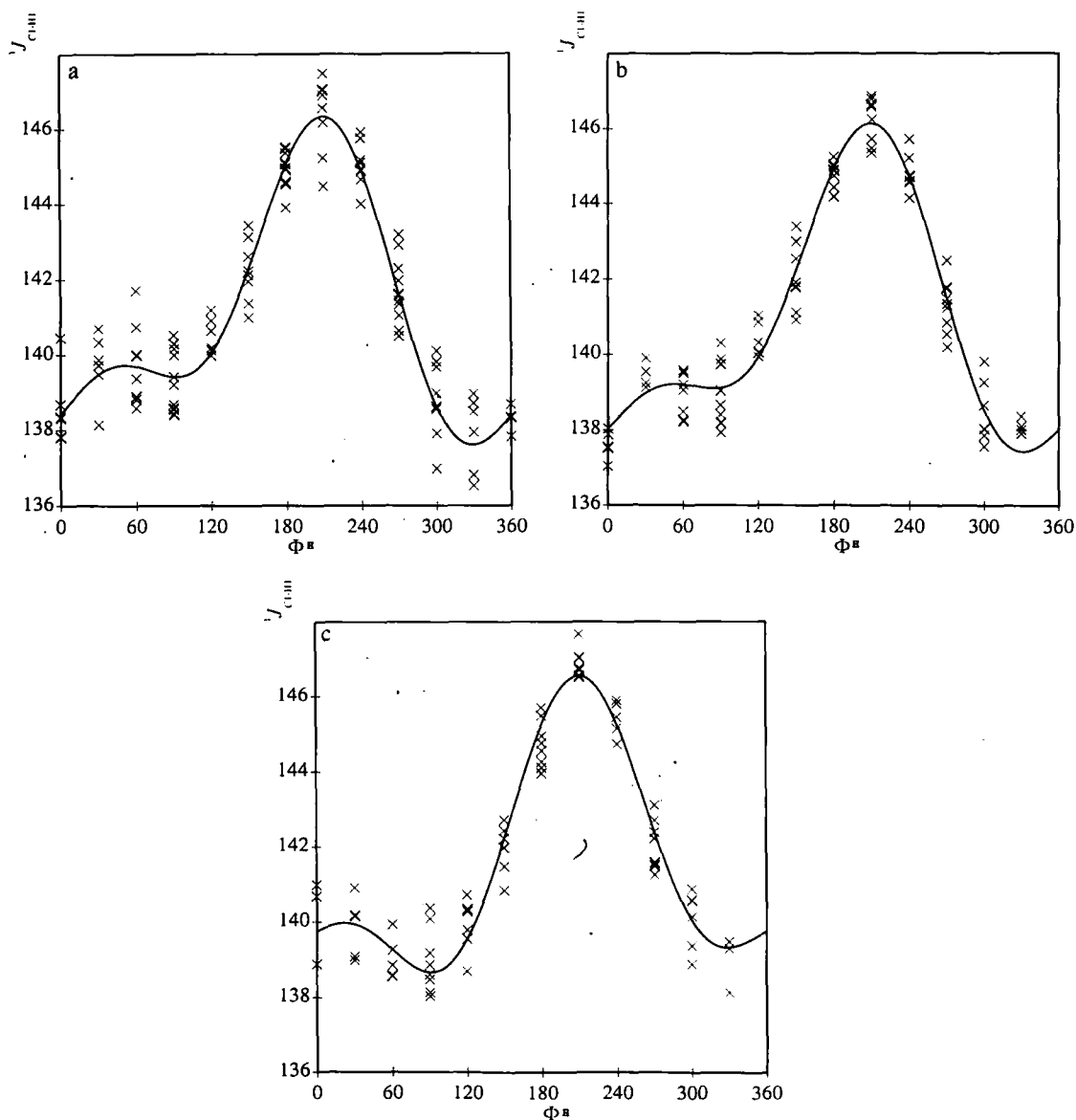Fig. 4. The $^1J_{C-1,H-1}$ values (X) as a function of Φ^H for: (a) 1e, (b) 2e, and (c) 3e. The solid curves show the best approximation to the indicated points.

Fig. 5. The $^1J_{C-X',H-X'}$ values (X) as a function of Ψ^H for: (a) 1e, (b) 2e, and (c) 3e. The solid curves show the best approximation to the indicated points.

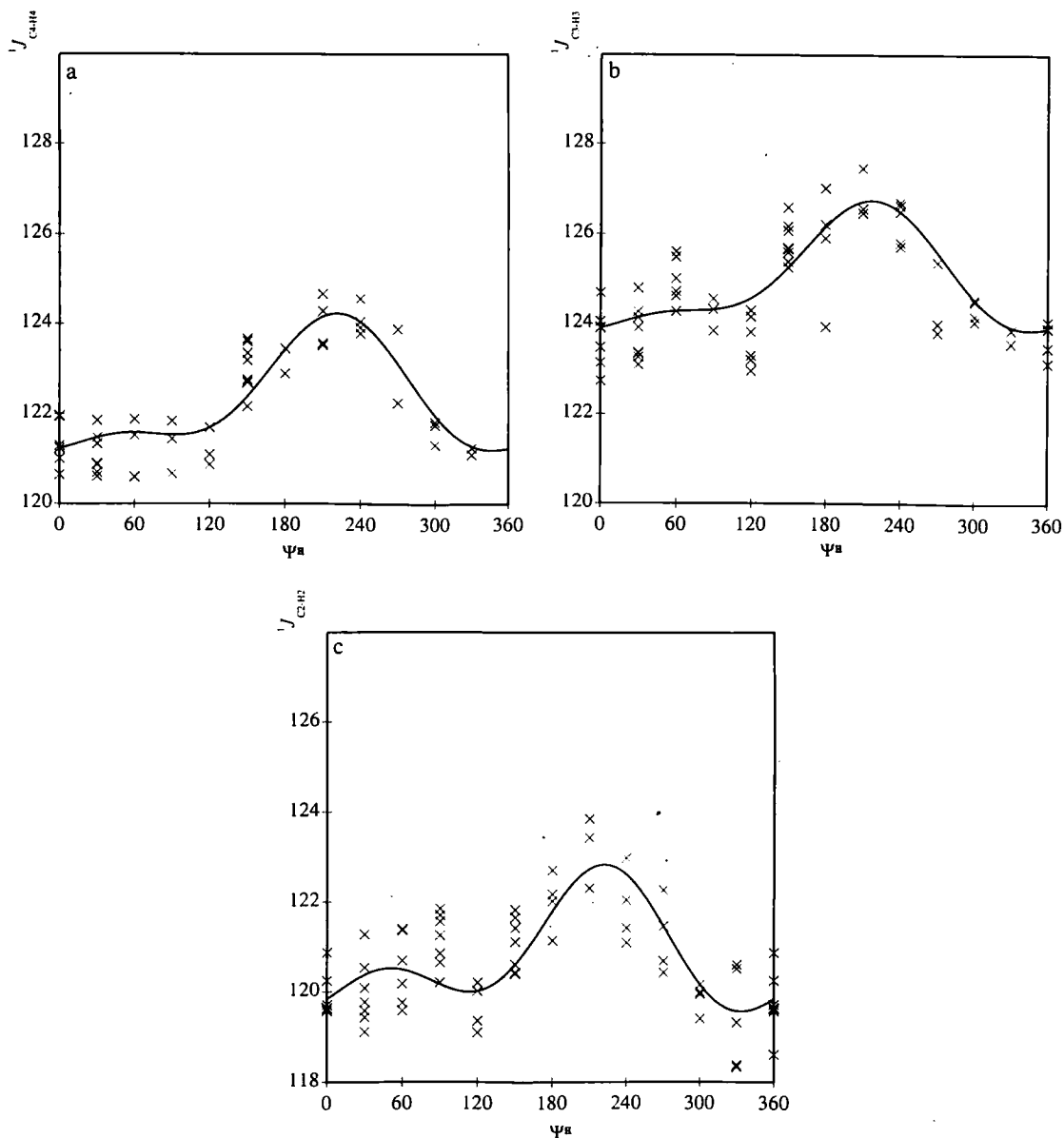As can be seen, the angular dependence of $^1J_{C-1,H-1}$ is similar for all types of molecules. Comparison with the dependence in α-linked models revealed a similarity between the angular dependence of $^1J_{C-1,H-1}$ in both types of molecules. In fact, the curves are mirror images with the plane of symmetry at Φ^H. A rationalization of the symmetrical relationship between the $^1J_{C-1,H-1}$ values in α-linked and β-linked models can be based on the inverse character of rotation about the anomeric C–O bond with the change of anomeric configuration. It is interesting to note that the calculated

TABLE 1

CALCULATED CONSTANTS A–E (Hz) OF Eq. 1 FOR $^1J_{C-1,H-1}$ IN **1e–6e**

Compound	A	B	C	D	E
1e	0.610	−3.328	1.710	−1.093	141.123
1e (118)	0.941	−4.725	1.703	−1.593	141.224
5e (g)	0.231	−3.192	1.814	−1.544	140.927
6e (m)	0.293	−3.417	1.281	−1.761	140.573
2e	0.600	−3.455	1.568	−1.184	140.816
3e	0.985	−2.747	1.422	−1.856	141.514
4e	0.145	−1.689	1.179	−0.963	141.573
Mean (1–4)[a]	0.519	−3.666	1.627	−1.498	140.951
Mean (total)[b]	0.610	−3.477	1.583	−1.505	141.023

[a] For a (1→4) linkage.

[b] The (1'→1) linkage was not included.

constants A, 0.31; B, −3.35; C, 1.91; and D, −1.70 for $^1J_{C-1,H-1}$ based only on methyl β-xylobio-side (Hricovini and Tvaroska, 1990) are in very good agreement with those in Table 1. Thus, the mean angular dependence of $^1J_{C-1,H-1}$, also taking into account the A–D values for methyl β-xylo-bioside, may be represented by the equation:

$$^1J_{C-1,H-1} = 0.57\cos 2\Phi^H - 3.46\cos \Phi^H + 1.63\sin 2\Phi^H - 1.53\sin \Phi^H + 141.75 \qquad (2)$$

The results calculated for $^1J_{C-X',H-X'}$ (Table 2) show that the angular dependence for all compounds is similar. However, a comparison with the dependence observed for α-linked models clearly shows that the effect of anomeric configuration on the angular dependence of $^1J_{C-X',H-X'}$ is not negligible. On the basis of these data alone, it is not possible to distinguish the individual electronic factors which lead to these differences. It might be connected with stronger delocalization interactions between lone pairs on oxygen atoms in β configuration than in α configuration (Tvaroska and Bleha, 1989). This suggests, in contrast to our observation in methyl xylobiosides (Hricovini and Tvaroska, 1990), that different angular dependences for $^1J_{C-X',H-X'}$ should be used for α- and β-linked oligosaccharides. These differences could not be observed in our previous investi-

TABLE 2

CALCULATED CONSTANTS A–E (Hz) OF Eq. 1 FOR $^1J_{C-X',H-X'}$ IN **1e–3e, 5e** AND **6e**

Compound	A	B	C .	D	E
1e	0.003	−1.096	0.564	−0.787	122.333
5e (g)	0.0	−0.367	−0.199	−1.585	123.093
6e (m)	0.014	−1.82	0.837	−1.402	124.256
2e	0.044	−1.131	0.485	−0.629	125.0
3e	0.001	−0.984	0.844	−0.654	120.826
Mean (1–4)	0.006	−1.094	0.401	−1.258	123.241
Mean (total)	0.0124	−1.080	0.506	−1.011	123.110

gation because of scattered data (Hricovini and Tvaroska, 1990). Thus, the mean angular dependence of $^1J_{C-X',H-X'}$ for β-linked models may be expressed in the form:

$$^1J_{C-X',H-X'} = 0.01 \cos 2\Psi^H - 1.08 \cos \Psi^H + 0.51 \sin 2\Psi^H - 1.01 \sin \Psi^H + 123.11 \qquad (3)$$

The greatest inadequacy of FPT-INDO descriptions of $^1J_{C,H}$ seems to be associated with the constant term, E. Therefore all of the ^{1}C,H values were adjusted by a constant amount using experimental data for molecules with a well-defined conformation. Unfortunately, in contrast to α-linked molecules and to our surprise, very few molecules in the β-configuration with well defined conformation are available. Therefore, we measured $^1J_{C,H}$ values for cellobiose (7) $^1J_{C-1,H-1}$ = 163.2 Hz and $^1J_{C-4',H-4'}$ = 147.3 Hz in water. For [O-β-D-glucopyranosyl (1→6)]₃ 1-6″ anhydride nonaacetate (9), the $^1J_{C-1,H-1}$ = 162 Hz in chloroform was taken from Bonas et al., (1991).

In our previous investigation (Tvaroska and Taravel, 1991) we found that for α-linked oligosaccharides the solvent effect on $^1J_{C,H}$ may be represented as a function of the dielectric constant (ε) of the solvent in the form:

$$^1J_{C-1,H-1} = 0.039 \, \varepsilon + {}^1J_{C-1,H-1} \text{ (in vacuum)} \qquad (4)$$

$$^1J_{C-X',H-X'} = 0.294 \, \varepsilon + {}^1J_{C-X',H-X'} \text{ (in vacuum)} \qquad (5)$$

Assuming that the same dependence may be applied to β-linked oligosaccharides, the experimental values were corrected for the solvent effect and compared with the corresponding calculated $^1J_{C,H}$ values based on Eqs. 2 and 3. The addition of the mean estimated differences between the calculated and experimental values (19.75 and 21.96 Hz) gives corrected values of E (161.5 and 145.1 Hz). Thus, taking into account solvent polarity, the angular dependence of $^1J_{C,H}$ is represented by the expressions:

$$^1J_{C-1,H-1} = 0.57 \cos 2\Phi^H - 3.46 \cos \Phi^H + 1.63 \sin 2\Phi^H - 1.53 \sin \Phi^H + 161.5 + 0.0390 \, \varepsilon \qquad (6)$$

$$^1J_{C-X',H-X'} = 0.01 \cos 2\Psi^H - 1.08 \cos \Psi^H + 0.51 \sin 2\Psi^H - 1.01 \sin \Psi^H + 145.1 + 0.0294 \, \varepsilon \qquad (7)$$

The experimental $^1J_{C,H}$ values in oligosaccharides reflect average values, in which the form of the conformational dependence is weighted with respect to the barrier that hinders internal rotation. Therefore, to compare the results based on equations 6 and 7 with the experimental values, it is necessary to average the available conformers, which requires knowledge of the conformational properties of a particular oligosaccharide in solution. For the estimation of proposed angular dependences we used mannobiose (8), methyl β-xylobioside (10), agarobiose (11) and neoagarobiose (12) for which conformational equilibria have been published (Tvaroska et al., 1987; Jimenez-Barbero et al., 1989; Hricovini et al., 1990).

In the case of 10, the PCILO calculation revealed the existence of seven minima for rotation about the glycosidic C-O bonds, but only six of them are present in the equilibrium mixture. The distribution of the conformers X1(Φ^H = 39.6°, Ψ^H = −67.4°), X2 (60.4, 48.4), X3(62.4, −152.4), X5(−35.9, −49.5), X6(118.9, −50.7), and X7(153.5, 29.9) in aqueous solution is 21.6:10.3:54.7:0.3:1.2:11.8 (Hricovini et al., 1990). The conformational averaging based on the above abundance of conformers and the $^1J_{C,H}$ values calculated using Eqs. 6 and 7 produced the

values $<^1J_{C-1,H-1}> = 162.4$ Hz and $<^1J_{C-4',H-4'}> = 147.6$ Hz. The magnitudes of the calculated $<^1J_{C,H}>$ values are in excellent agreement with the observed experimental values of 162.7 Hz and 147.5 Hz (Hricovini and Tvaroska, 1990). It is interesting to note that this agreement is better than for the values of 163.7 Hz and 146 Hz obtained using the relationship for compound **10** only (Hricovini and Tvaroska, 1990).

The MM2CARB calculations predicted (Jimenez-Barbero et al., 1989) the five neoagarobiose conformers: GA1 ($\Phi^H = -70.8°$, $\Psi^H = -75.5°$), GA2 (39.3, −62.5) GA3 (53.1, 42.0), GA4 (172.4, −17.3) and GA5 (124.9, −60.8) in the ratios 0.1:8.5:85.0:4.0:2.4; and the six agarobiose conformers: AG1 ($\Phi^H = 53.3°$, $\Psi^H = -42.9°$), AG2 (40.0, −61.9), AG3 (37.8, 159.9), AG4 (52.6, 23.8), AG5 (163.4, 5.3) and AG6 (64.8, 46.1) in the ratios 0.8:81.7:1.2:1.9:13.9:0.5. Averaging based on the above abundance of conformers and the $^1J_{C,H}$ values calculated using Eqs. 6 and 7 produced the values $<^1J_{C-1,H-1}> = 162.8$ Hz and $<^1J_{C-4',H-4'}> = 146.6$ Hz for neoagarobiose and $<^1J_{C-1,H-1}> = 163.2$ Hz and $<^1J_{C-3',H-3'}> = 147.3$ Hz for agarobiose. The values at the anomeric center can be compared to the experimental values $^1J_{C,H}$ 160.9 Hz and 165.8 Hz in aqueous solution for the basic trisaccharide unit of agarose which consists of alternating 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro α-L-galactopyranose. Since the conformational equilibria used for calculations of $<^1J_{C,H}>$ were estimated for disaccharide repeating units of agarose, i.e. agarobiose and neoagarobiose, the agreement is satisfactory.

The calculation for mannobiose (Tvaroska et al., 1987) showed that of the seven conformers found, only five are present in the equilibrium mixture of **8** in aqueous solution. The ratios of the conformers M1 ($\Phi^H = 23.9°$, $\Psi^H = 63.8°$), M2 (32.3, 30.2), M3 (112.1, −29.0), M4 (48.4, 31.0), and M7 (25.4, −46.0) were 4.1:4.2:86.9:0.8:4.0. The calculated values $<^1J_{C-1,H-1}> = 162.4$ Hz and $<^1J_{C-4',H-4'}> = 147.6$ Hz can be compared to the experimental values $<^1J_{C-1,H-1}> = 160.4$ Hz and $<^1J_{C-4',H-4'}> = 147.2$ Hz.

The results presented in this and previous papers (Tvaroska, 1990; Hricovini and Tvaroska, 1990; Tvaroska and Taravel, 1991) indicate the reliability of the approach used to establish the relationship between dihedral angle and $^1J_{C,H}$ values for α- and β-linked oligosaccharides. This approach consists of a combination of FPT INDO MO calculations and experimental data of the angular dependence of $^1J_{C,H}$ for compounds with a well defined conformation around the glycosidic linkage. The comparison with available experimental data suggest that the proposed curves describing the angular dependence of $^1J_{C,H}$ can be utilized to determine the conformation of glycosidic linkage in solution.

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