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Carbohydrate Research 309 (1998) 145–152

CARBOHYDRATE RESEARCH

Two-bond ^{13}C–^{13}C spin-coupling constants in carbohydrates: New measurements of coupling signs

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Received 19 February 1998; accepted 23 April 1998

Abstract

D-(1,3,6-$^{13}C_3$)Allose (**1**), (^{13}C)methyl α-D-(1,2-$^{13}C_2$)glucopyranoside (**2**) and (^{13}C)methyl β-D-(1,2-$^{13}C_2$)glucopyranoside (**3**) were synthesized and used to establish the signs of their constituent $^2J_{CCC}$ or $^2J_{COC}$ values ($^2J_{C1,C3}$ in the α-pyranose of **1** (**15**), and $^2J_{C1,CH_3}$ in **2** and **3**). Compounds **2**, **3** and **15** contain three mutually coupled labeled carbons, thus creating a three-spin system from which crosspeak displacements in ^{13}C–^{13}C COSY-45 spectra were used to determine coupling signs. In all compounds, at least one $^3J_{CC}$ value was present as an internal reference: $^3J_{C2,CH_3}$ in **2** and **3**, and $^3J_{C1,C6}$ and $^3J_{C3,C6}$ in **15**. $^2J_{C1,CH_3}$ in **2** and **3**, and $^2J_{C1,C3}$ in **15**, were found to be negative, thus providing experimental confirmation of the sign predictions made via the projection resultant rule described recently.

Keywords: $^2J_{COC}$; $^2J_{CCC}$; ^{13}C-^{13}C COSY-45; spin-coupling signs; projection resultant method

1. Introduction

In recent years considerable discussion about the conformational properties of oligosaccharides has focused on the flexibility of their constituent glycosidic linkages [1]. Much of this discussion, however, can be misleading, since it is likely that the flexibility of a given linkage will depend on its context, that is, on the specific structural environment in which the linkage is found. The term 'flexibility' is hierarchical; in reality, all linkages will experience some degree of motion or libration. The key question is the amplitude of this motion, that is, the extent of the excursions experienced by a given torsion angle. These questions are frequently addressed experimentally by exploiting ^1H–^1H nuclear Overhauser effects (NOE) between residues, but significant, and often overlooked, problems accompany this approach. Due to the r^{-6} dependence of the NOE, the interpretation of NOEs in structural terms is complicated when conformational flexibility exists, since the averaging of NOEs is non-linear, as discussed by Jardetzky [2]. NOEs will be skewed in favor of those conformers containing the more potent relaxation pathways (i.e., the shorter internuclear distances) even when they are present in minor abundance, as

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0008-6215/98/$—see front matter
PII S0008-6215(98)00123-2

clearly explained by Homans [3]. This limitation is overcome by maximizing the number of NOEs used in the analysis. Unfortunately, in many oligosaccharides, the number of NOEs that are sensitive to a given linkage geometry is limited. Thus, despite its widespread use, NOE is not, on its own, capable of resolving the issue of conformational flexibility in oligosaccharides in a unequivocal fashion.

Additional information on O-glycoside linkage conformation can, in principle, be derived from the analysis of J_{CH} and J_{CC} values across these linkages [4]. For the linkage shown in Fig. 1, two $^{3}J_{COCH}$, one $^{2}J_{COC}$ and three $^{3}J_{COCC}$ values are available, and unlike NOE, these scalar couplings should, in principle, be interpreted reliably in the presence of conformational averaging [2,5]. Importantly, the ϕ and ψ torsion angles (Fig. 1) can each be assessed independently by three couplings, yielding data redundancy which improves the accuracy of the analysis.

A prerequisite in the use of the trans-O-glycoside couplings shown in Fig. 1 is a detailed understanding of how they are influenced by molecular structure. Mulloy et al. [6] and Tvaroska et al. [7] have proposed virtually identical Karplus curves for the treatment of $^{3}J_{COCH}$ values, but $^{2}J_{COC}$ and $^{3}J_{COCC}$ values in carbohydrates remain poorly understood. In response to the latter, we proposed recently a projection resultant method to predict the magnitudes and signs of $^{2}J_{CCC}$ and $^{2}J_{COC}$ values in carbohydrates [8], and some experimental verification of the method has been reported [9a]. In the present study, we provide new verification of the method through the experimental determination of $^{2}J_{CC}$ coupling signs using multiply ^{13}C-labeled compounds and the ^{13}C–^{13}C COSY-45 method [9b].

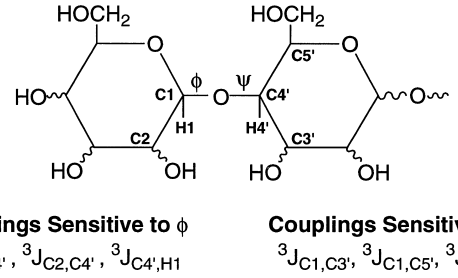
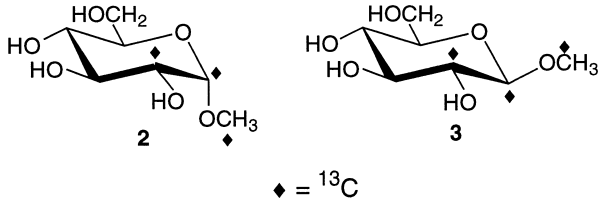


Fig. 1. Trans-O-glycoside J_{CH} and J_{CC} sensitive to the glycosidic torsional angles, ϕ and ψ.

2. Experimental

Synthesis of ^{13}C-labeled compounds.—D-(1,3,6-$^{13}C_3$)-Allose (**1**), (^{13}C)methyl α-D-(1,2-$^{13}C_2$)glucopyranoside (**2**) and (^{13}C)methyl β-D-(1,2-$^{13}C_2$)glucopyranoside (**3**) were prepared and purified by methods reported previously, and only a brief account of their synthesis is reported here.

The preparation of **1** is outlined in Scheme 1. D-(6-^{13}C)Glucose, prepared from 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose and K^{13}CN (99 atom% ^{13}C, Cambridge Isotope Laboratories) as described by King-Morris et al. [10], was oxidized with $Pb(OAc)_4$ to give D-(4-^{13}C)erythrose (**4**) [11]. Compound **4** was converted to D-(1,5-$^{13}C_2$)arabinose (**5**) and D-(1,5-$^{13}C_2$)ribose (**6**) by cyanohydrin reduction [12,13], and the mixture was purified by chromatography on Dowex-50×8 (200–400 mesh) in the Ca^{+2} form [14,15]; compound **5** eluted first, followed much later by compound **6** [16]. Compound **5** was converted to D-(2,5-$^{13}C_2$)ribose (**7**) by molybdate-catalyzed epimerization [17]. Subsequent cyanohydrin reduction applied to **7** afforded pure **1** after chromatography on Dowex-50×8 (200–400 mesh) in the Ca^{+2} form [14,15]; the C-2 epimer, D-(1,3,6-$^{13}C_3$)altrose (**8**), eluted first, followed by **1**. Compound **1** was identified by its characteristic ^{13}C chemical shifts [18].



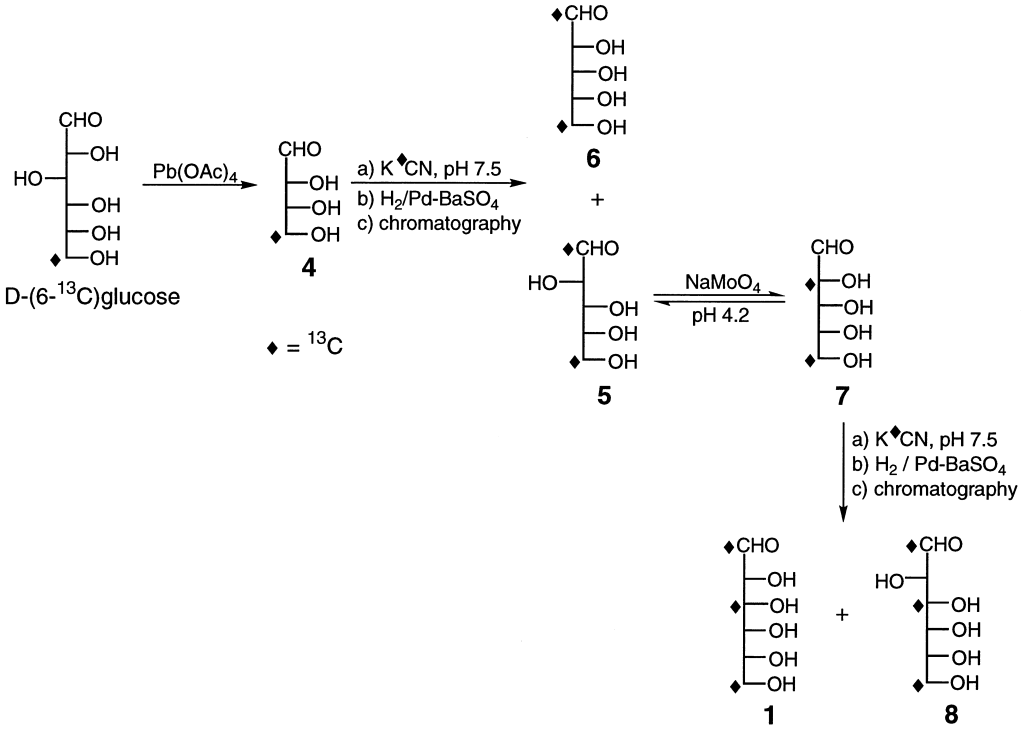
(^{13}C)Methyl α- (**2**) and β- (**3**) D-(1,2-$^{13}C_2$)glucopyranosides were prepared from (^{13}C)methanol and D-(1,2-$^{13}C_2$)glucose [15] by Fischer glycosidation using THF as a cosolvent and Dowex-50 H^+ ion-exchange resin as the catalyst [19] according to the following procedure. HCR-W2 (H^+) ion-exchange resin (Sigma Chemical Co.), washed with water and dried under vacuum overnight, was added to a 10-mL round-bottomed flask containing D-(1,2-$^{13}C_2$)glucose (0.36 g, Omicron Biochemicals), and (^{13}C)methanol (0.5 mL, Aldrich Chemical Co.) and anhydrous THF (3 mL) were added. The mixture was refluxed for 2 h under nitrogen, cooled, and filtered to remove the resin. The recovered resin was washed with 2-propanol, and the combined filtrate and washings were

concentrated in vacuo to give a colorless syrup (~0.39 g). The syrup was dissolved in a minimum volume of water, and the solution was applied to a column (2.5 cm×60 cm) of Dowex-1×2 (200–400 mesh) ion-exchange resin in the OH^- form [20a]. The column was eluted with water, and fractions (18 mL) were collected and assayed for sugar with phenol–sulfuric acid [20b]. Fractions 24–28 and 32–35 were pooled and concentrated in vacuo to give pure (^{13}C)methyl α-D-(1,2-$^{13}C_2$)glucopyranoside (**2**) (0.15 g) and (^{13}C)methyl β-D-(1,2-$^{13}C_2$)glucopyranoside (**3**) (0.07 g), respectively, as syrups. Compounds **2** and **3** were identified by their characteristic ^{13}C chemical shifts [18].

NMR spectroscopy.—NMR spectra of **2** and **3** (~100 mM in ^2H_2O, 5 mm tubes) were obtained at 30 °C on a Varian Unity*Plus* NMR spectrometer (599.89 MHz ^{1}H, 150.85 MHz ^{13}C). 2D ^{13}C–^{13}C COSY data sets were collected in the absolute intensity mode using pulse sequences described previously [21]. The WALTZ scheme of broadband decoupling was used to enhance the intensity of the detected carbon signals through NOE. The t_1 increments (3072) of eight scans each were sampled in 4096 data points, and zero-filling to 4 and 8 K was applied in the F_1 and F_2 dimensions, respectively. Sinebell weighting functions (determined empirically) were applied in both dimensions prior to double Fourier transformation. ^{13}C Chemical shifts are reported in ppm, and are referenced to the C-1 chemical shift of α-D-mannopyranose (95.5 ppm).

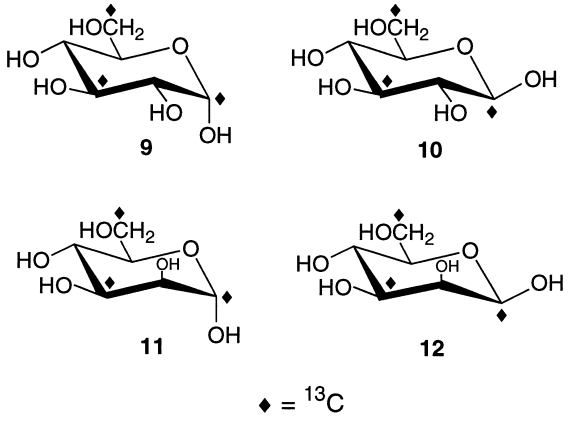
3. Results

Definition of the problem.—In a previous report [8], we proposed that the magnitude and signs of $^2J_{CCC}$ values could be correlated with an empirically derived projection resultant, with the latter determined by inspection of two rotamer projections along the molecular fragment containing the coupling constant under consideration. Using a range of standard compounds, these resultants were directly correlated with $^2J_{CCC}$ magnitude and sign, with more positive resultants yielding more positive couplings. For example, β-D-glucopyranose and α-D-allopyranose yield projection resultants of +2.5 and −0.5, respectively, and $^2J_{C1,C3}$ values of +4.5 and −2.4 Hz are predicted in these aldohexoses (Fig. 2). This correlation was then tested experimentally by determining the signs of $^2J_{C1,C3}$ in α- (**9**) and β- (**10**) D-(1,3,6-$^{13}C_3$)glucopyranoses and α- (**11**) and β- (**12**) D-(1,3,6-$^{13}C_3$)mannopyranoses from crosspeak displacements in ^{13}C–^{13}C COSY-45 spectra [9a]. These experiments confirmed that $^3J_{C1,C3}$ in **10**



Scheme 1.

(+4.5 Hz) and **12** (+4.0 Hz) were positive as predicted ($^2J_{C1,C3} = \sim 0$ Hz in **9** and **11**). To date, however, experimental verification of *negative* $^2J_{CCC}$ values (e.g., $^2J_{C1,C3}$ in α-D-allopyranose, Fig. 2) has not been reported, although the method predicts the existence of such couplings for resultants less than ~1. Observation of negative $^2J_{CCC}$ values consistent with predictions would provide a key validation of the method.



The treatment of $^2J_{CCC}$ values was extended to $^2J_{COC}$ values, where access to model compounds is considerably more limited. Projection resultants calculated for $^2J_{C1,C5}$ in D-aldopyranoses were more *positive* for β anomers than for α anomers, and by analogy to $^2J_{CCC}$ behavior, $^2J_{C1,C5}$ in α anomers was predicted to be more *negative* (less positive) than $^2J_{C1,C5}$ in β anomers. Since $^2J_{C1,C5}$ is ~0 Hz in β anomers and ~2.0 Hz in α anomers [22], the latter couplings were predicted to be negative, and this prediction has been confirmed experimentally via ^{13}C–^{13}C COSY-45 analysis of α-(**13**) and β- (**14**) D-(1,5,6-$^{13}C_3$)mannopyranoses [9a]. Importantly, the extrapolation of these results to $^2J_{COC}$ behavior across O-glycosidic linkages allowed for the prediction of $^2J_{COC}$ behavior in two of the three rotamers that relate to φ (rotamers A and C, Fig. 3), namely, those containing C–O–C coupling pathways structurally related to those found in the α- and β-D-aldopyranoses; since no projection is derived from ψ, this torsion does not appear to influence the value of $^2J_{COC}$ significantly [8]. No model compound, however, was available at that time to evaluate the third rotamer (rotamer B, Fig. 3). The projection method predicts a negative coupling in rotamer B, and this prediction is tested in this report.

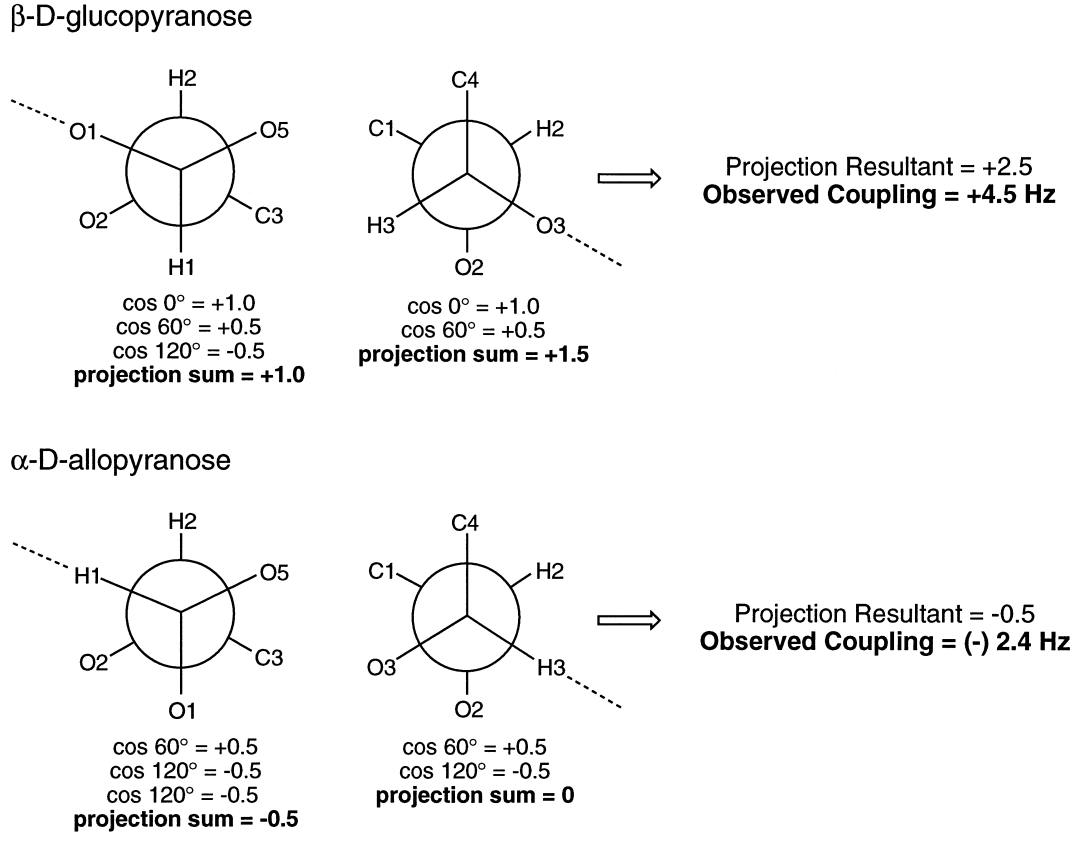
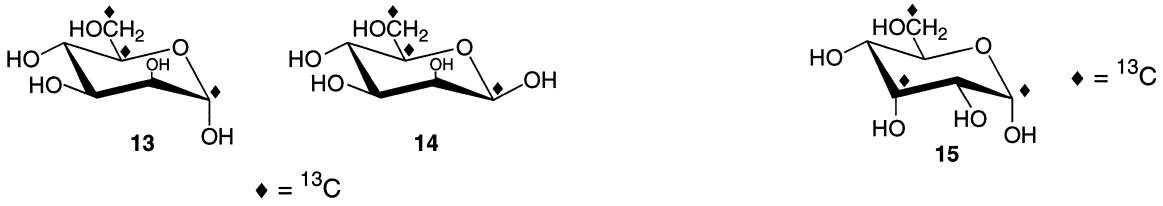


Fig. 2. Correlation between projection resultants and $^2J_{C1,C3}$ in β-D-glucopyranose and α-D-allopyranose.



New $^2J_{CCC}$ *and* $^2J_{COC}$ *sign determinations.*—The ^{13}C-labeling pattern in D-(1,3,6-$^{13}C_3$)allose (**1**) creates a mutually-coupled three-spin system in α-D-(1,3,6-$^{13}C_3$)allopyranose (**15**) involving three J_{CC} values [22b,23], namely, $^2J_{C1,C3}$ (2.4 Hz), $^3J_{C3,C6}$ (2.8 Hz) and $^3J_{C1,C6}$ (3.2 Hz). This three-spin system is required in order to determine coupling signs straightforwardly via ^{13}C–^{13}C COSY-45 [9a]. The two three-bond (vicinal) couplings are expected to be positive in sign [24]. Consequently, the crosspeak displacements observed for the C-1–C-6 and C-3–C-6 crosspeaks should have the same relative orientation and can be correlated with a positive coupling. The sign of $^2J_{C1,C3}$ can be established by comparing these standard displacements with that observed for the C-1–C-3 crosspeak (i.e., same relative displacement, + sign; different relative displacement, − sign).

The C-1–C-6, C-3–C-6 and C-1–C-3 ^{13}C–^{13}C COSY-45 crosspeaks for **15** are shown in Fig. 4. The C-1–C-6 and C-3–C-6 crosspeaks show the same relative displacements as expected, since both couplings are positive. However, the C-1–C-3 crosspeak shows the opposite displacement, thus indicating that $^2J_{C1,C3}$ is *negative*, which confirms the projection resultant prediction.

As in **15**, a mutually coupled three-spin system is present in (^{13}C)methyl α- (**2**) and β- (**3**) D-(1,2-$^{13}C_2$)glucopyranosides involving $^1J_{C1,C2}$, $^2J_{C1,CH_3}$ and $^3J_{C2,CH_3}$. The C-2–CH_3 and C-1–CH_3 crosspeaks in the ^{13}C–^{13}C COSY-45 spectrum of **3** show opposite displacements (Fig. 5). Since the relative displacement observed for the three-bond pathway is correlated with a positive coupling, $^2J_{C1,CH_3}$ must be *negative* in sign. Similar data were obtained on **2** (data not shown). These results confirm the projection rule predictions made earlier for rotamer B in Fig. 3 [8].

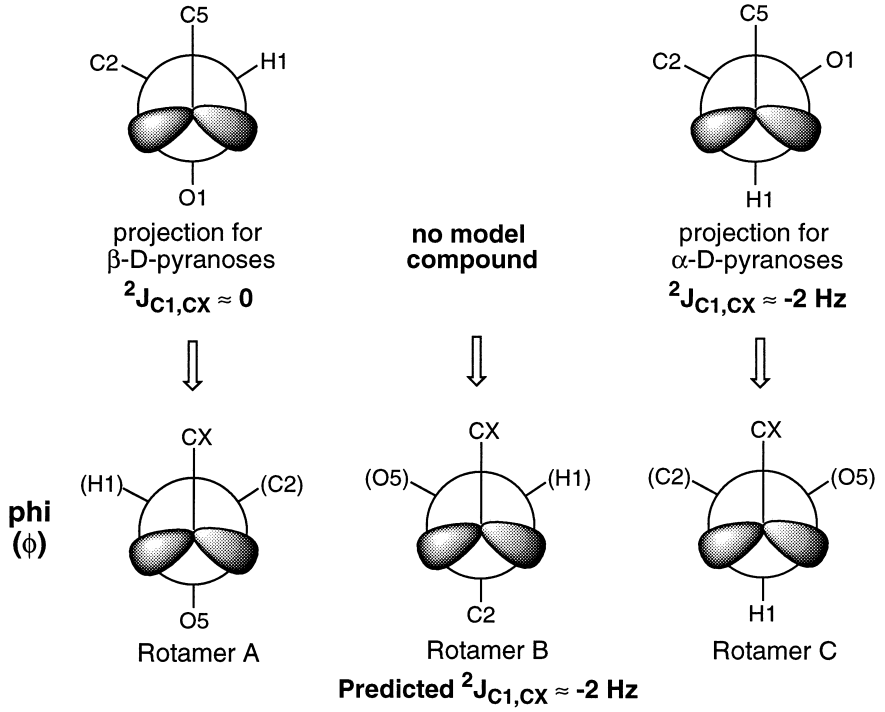


Fig. 3. Correlation between projection resultants and trans-O-glycoside $^2J_{COC}$ derived from model studies of $^2J_{C1,C5}$ in α- and β-D-aldopyranoses.

4. Discussion

The results of this investigation provide new experimental validation of the projection resultant method for the prediction of $^{2}J_{CCC}$ and $^{2}J_{COC}$ values in carbohydrates. The negative sign of $^{2}J_{C1,C3}$ in **15**, and the negative signs of $^{2}J_{C1,CH_3}$ in **2** and **3**, fulfill the predictions and provide critical tests of the method. In **2** and **3**, the exoanomeric effect [25–27] stabilizes the C-1–O-1 rotamer having O-1 *gauche* to H-1 and O-5 (rotamer B in Fig. 3), and this preferred orientation is supported by the relatively large $^{2}J_{C2,CH_3}$ values observed in these structures (e.g., 3.0 Hz in **2**) and the magnitudes of $^{3}J_{CH_3}$,H1 values reported previously by Lemieux and Koto in methyl aldopyranosides (3.2–4.6 Hz) [28]. Thus, while the presence of rotamers A and C (Fig. 3) cannot be excluded in solutions of **2** and **3**, their contributions to the observed couplings are expected to be small.

The origin of the displacements observed in COSY-45 spectra, from which coupling signs are

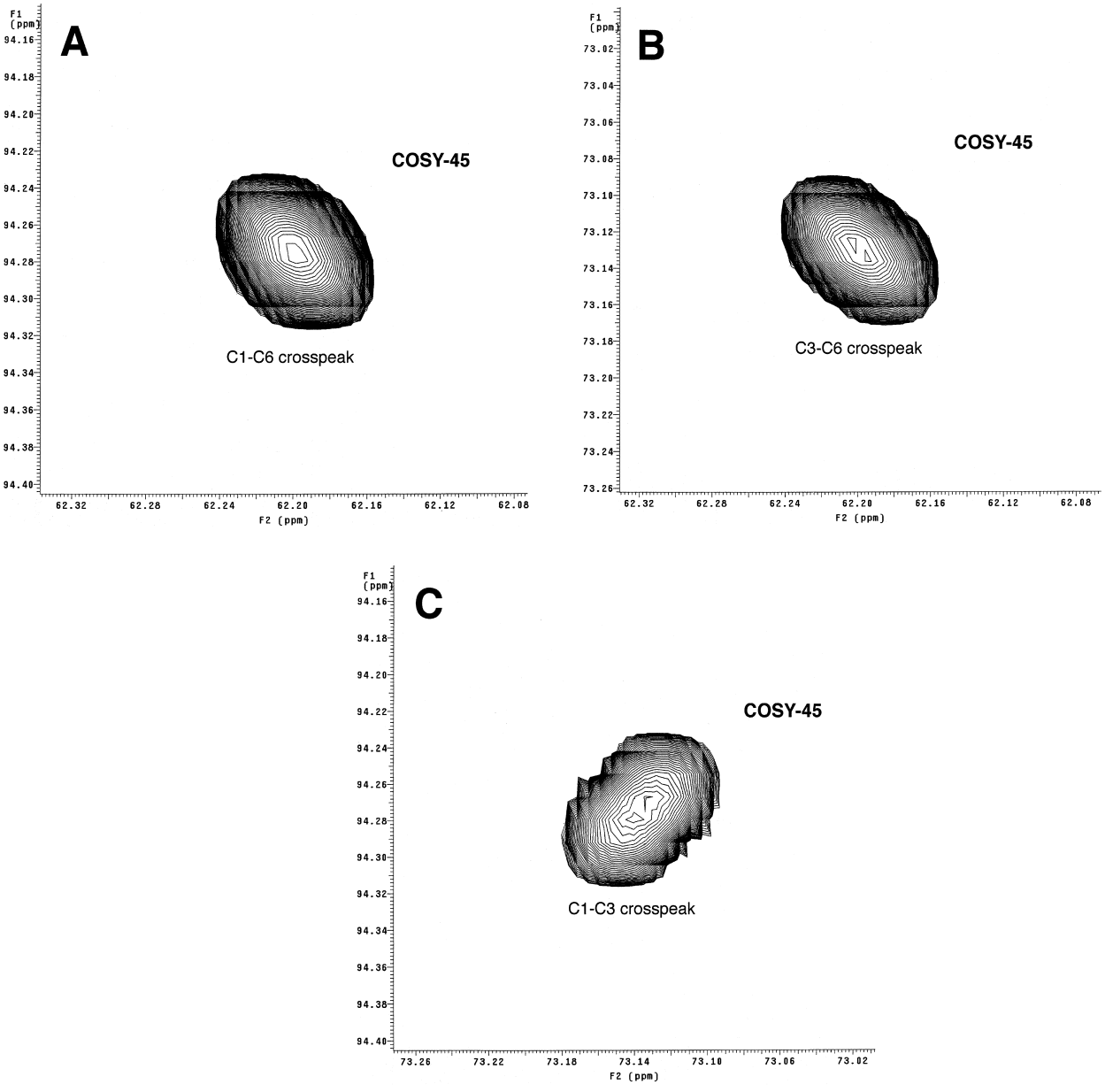


Fig. 4. Crosspeaks observed in the ^{13}C–^{13}C COSY-45 spectrum of **15**. (A) C-1–C-6 crosspeak. (B) C-3–C-6 crosspeak. (C) C-1–C-3 crosspeak. The relative displacement observed in (C) differs from that observed in (A) and (B), indicating that the sign of $^{2}J_{C1,C3}$ (negative) is opposite to that of $^{3}J_{C1,C6}$ and $^{3}J_{C3,C6}$ (positive).

determined, has been explained previously [5,29]. The structures of the crosspeaks for **15** (Fig. 4) differ from those observed in **3** (and **2**) (Fig. 5) in that the large $^{1}J_{C1,C2}$ is absent in the former. This difference makes the detection of the displacement in the COSY-45 data for **15** more difficult, but nevertheless observable. Crosspeak displacements as small as ~2 Hz were readily observed in this study, and a lower limit for the detection of displacements is estimated to be 1–1.5 Hz when a large $^{1}J_{CC}$ is absent (e.g., **15**), and 0.5–1 Hz when $^{1}J_{CC}$ splits the crosspeak (e.g., **2** and **3**).

The values of $^{2}J_{COC}$ across O-glycosidic linkages range from 0 Hz to ~−2 Hz (Fig. 3), and appear to depend primarily on the phi (ϕ) torsion angle [8] (Fig. 1). Thus, *when used in conjunction with other structural information* (e.g., NOE, $^{3}J_{COCC}$, $^{3}J_{COCH}$), they may improve the analysis of the conformational properties of oligosaccharides. In some instances, torsional motion about these linkages might be significant, leading to the averaging of the observed coupling. Given the relatively small expected range of values (~2 Hz), some limitations might accrue from the use of these couplings in these instances. However, recent reports have shown that J_{CC} values as low as 0.1–0.2 Hz can be measured reliably in biomolecules [30], including small proteins, and the application of these methods to oligosaccharides should permit a meaningful interpretation of $^{2}J_{COC}$ values in conformationally flexible molecules.

In conclusion, this study demonstrates the utility of triply ^{13}C-labeled compounds and the ^{13}C–^{13}C COSY-45 method in the determination of ^{13}C–^{13}C spin-coupling signs in saccharides, and has provided further experimental support of the projection

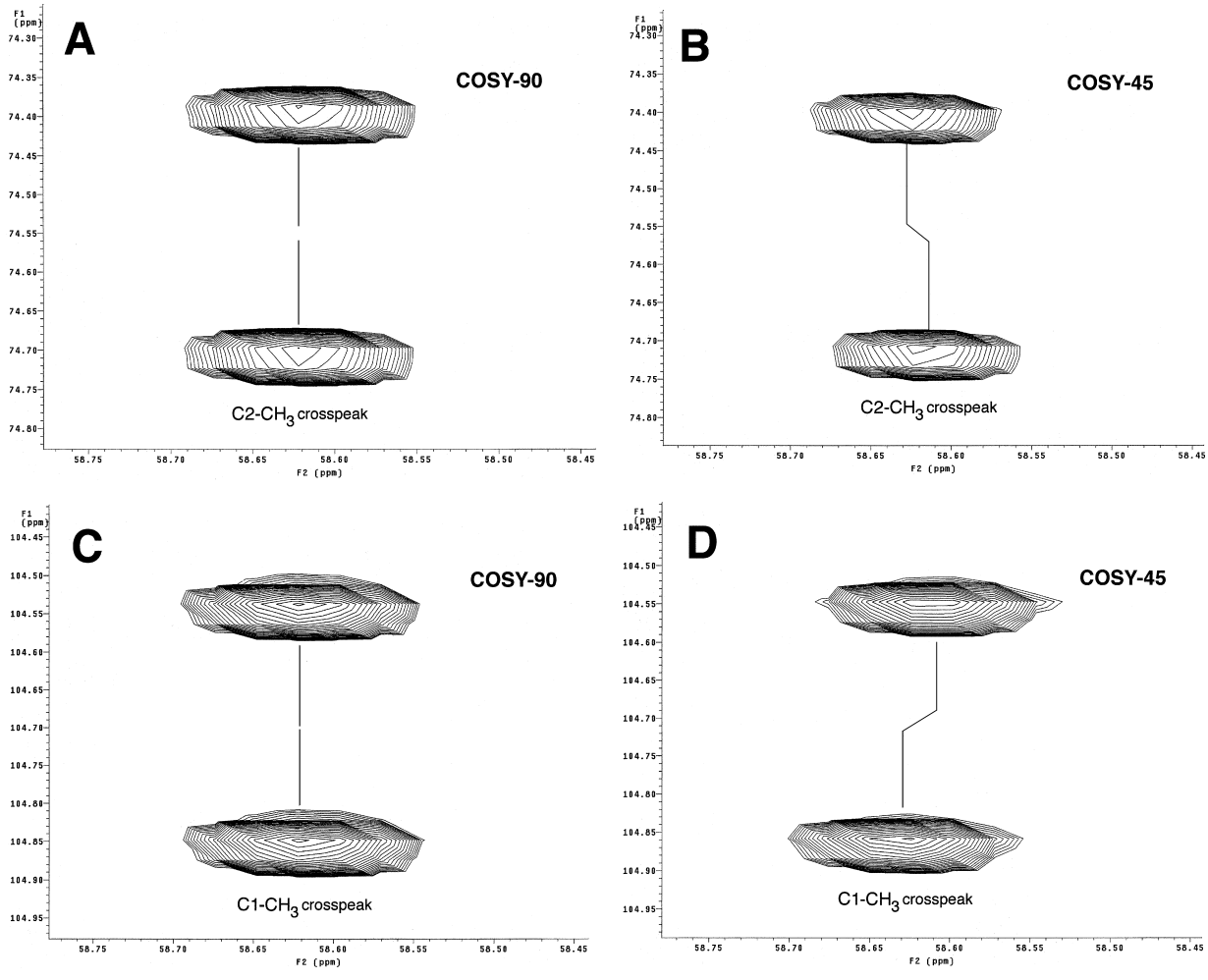


Fig. 5. Crosspeaks observed in the ^{13}C–^{13}C COSY-90 and ^{13}C–^{13}C COSY-45 spectra of **3**. (A) C-2–CH_3 crosspeak, COSY-90. (B) C-2–CH_3 crosspeak, COSY-45. (C) C-1–CH_3 crosspeak, COSY-90. (D) C-1–CH_3 crosspeak, COSY-45. No displacements are expected in the COSY-90 data, which serve as controls. The opposite relative displacements observed in (B) and (D) indicate that $^{3}J_{C2,CH_3}$ and $^{2}J_{C1,CH_3}$ have opposite signs (positive and negative, respectively).

resultant method for predicting the magnitudes and signs of $^{2}J_{CC}$ values in these molecules. Future work will extend the use of these coupling constants, and $^{3}J_{COCC}$ values (Fig. 1), to studies of the conformational properties of the O-glycosidic linkages in ^{13}C-labeled oligosaccharides.

Acknowledgement

Financial support of this work by Omicron Biochemicals, Inc. of South Bend, IN, is gratefully acknowledged.

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