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Measurement of inter-glycosidic ^{13}C–^{1}H coupling constants in a cyclic β(1→2)-glucan by ^{13}C-filtered 2D {^{1}H,^{1}H}ROESY

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SUMMARY

A method for measuring three-bond ^{13}C–^{1}H scalar coupling constants across glycosidic bonds in a cyclic β(1→2)-glucan icosamer is presented. This oligosaccharide molecule, with its high degree of symmetry, represents a particular challenge for NMR spectroscopy to distinguish inter-residue from intra-residue heteronuclear coupling effects. Chemically equivalent H2 protons in adjacent glucosyl residues are distinguished on the basis of their different through-space, dipolar interactions with the anomeric protons (H1). The strong NOE contact between anomeric (H1) and aglyconic (H2′) protons permits the selective observation of the inter-residue heteronuclear couplings $^{3}J_{C1H2'}$ and $^{3}J_{C2'H1}$ in a natural-abundance ^{13}C-ω_1-half-filtered {^{1}H,^{1}H} ROESY experiment.

INTRODUCTION

Cyclic β(1→2)-D-glucans produced by gram-negative bacteria are thought to play an important role in adapting these organisms to changes in environmental osmotic pressure by regulating the osmotic balance between the cytoplasm and the periplasmic space (Miller et al., 1986). NMR spectroscopic analysis is used in our laboratories to determine the three-dimensional structures of these cyclic glucans, in an attempt to provide insight into their intriguing physicochemical properties. Specifically, the magnitudes of three-bond ^{13}C–^{1}H scalar couplings across the glycosidic linkages are sought because they supply unique information about the distribution of the torsional bond angles, Φ and Ψ, in oligosaccharides (Poppe et al., 1992). However, cyclic β(1→2)-glucans pose a special problem for inter-glycosidic $^{3}J_{CH}$ measurements.

The ^{1}H-NMR spectrum of a cyclic β(1→2)-glucan icosamer (i.e., the oligosaccharide that

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Abbreviations: COSY, scalar correlated spectroscopy; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; ROESY, rotating-frame NOE spectroscopy.

contains 20 β-glucopyranose units, as schematically drawn in Fig. 1) resembles that of a single β-glucose molecule. The simplicity of this spectrum is a manifestation of the high degree of symmetry of the molecule, at least on the time scale of an NMR experiment. All constituent glycosyl residues are chemically equivalent. It is readily appreciated that most methods available for tracing long-range heteronuclear scalar connectivities would not distinguish intra-residue ($^2J_{C1H2}$ and $^2J_{C2H1}$) from inter-residue ($^3J_{C1H2'}$ and $^3J_{C2'H1}$) coupling effects. Indeed, recently proposed schemes for the measurement of inter-glycosidic $^3J_{CH}$ coupling constants (Poppe and Van Halbeek, 1991a,b; Lerner and Adams, 1992; Uhrín et al., 1992) are bound to fail for cyclic β(1→2)-glucans because these methods are based on coherence transfer mediated by long-range ^{13}C–^1H scalar coupling. Consequently, these methods do not make it possible to distinguish between the scalar couplings of a single ^{13}C-nucleus (for example, C1) and two different but chemically equivalent protons (H2 and H2′). For the measurement of the sought-after inter-glycosidic $^3J_{CH}$ values in the cyclic glucan icosamer, we resorted to a class of methods that provide

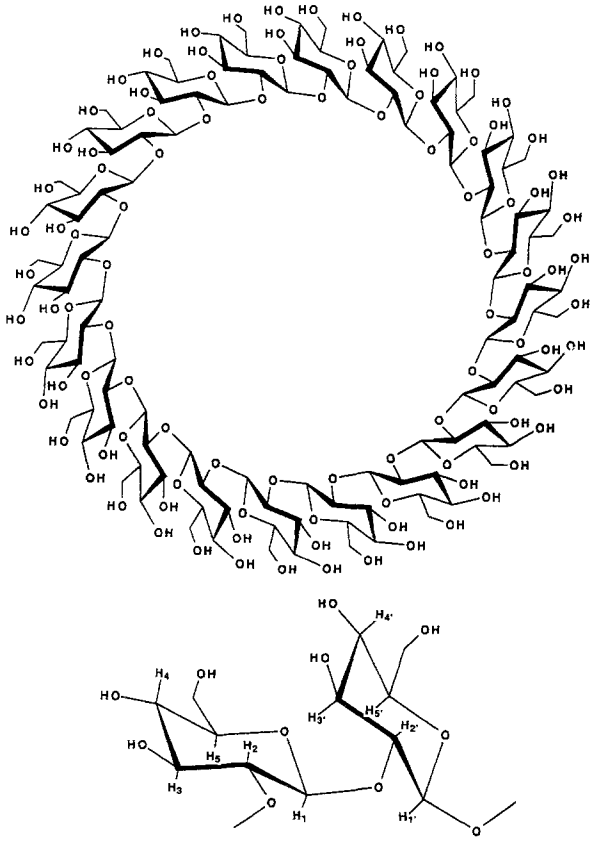


Fig. 1. Structure of the cyclic β(1→2)-glucan icosamer. Throughout this article, when specifying inter-nuclear interactions, a prime is placed beside the number of a nucleus to indicate that the nucleus resides in the glucosyl residue adjacent to the one with which it interacts (e.g., $^3J_{C1H2'}$). As all residues in the cyclic glucan isocamer are chemically equivalent, $^3J_{C1H2'}$ is identical to $^3J_{C1'H2}$. No primes are used when specifying interactions between numbered nuclei within the same residue (e.g., $^3J_{H1H2}$ and $^2J_{C1H2}$) or when generalizing interactions (e.g., $^3J_{CH}$).

^{13}C-filtered {^{1}H,^{1}H} correlation spectra in which long-range heteronuclear scalar coupling effects are observable (Montelione et al., 1989). These methods proved invaluable for measuring long-range heteronuclear scalar couplings in isotopically enriched proteins (Wider et al., 1989; Edison et al., 1991) and have also been successfully applied to polypeptides with a natural abundance of ^{13}C (Otting and Wüthrich, 1990; Schmieder et al., 1991; Sattler et al., 1992).

In oligosaccharides, protons that are directly attached to the carbons involved in a glycosidic linkage typically show a relatively strong NOE interaction (Bush, 1988). This is the case for the cyclic β(1→2)-glucan depicted in Fig. 1, resulting in a significantly (about seven times) stronger NOE interaction for inter-residue H1–H2′ pairs than for intra-residue, transdiaxial H1–H2 pairs. Consequently, selective observation and measurement of the inter-glycosidic scalar coupling constants $^{3}J_{C1H2'}$ and $^{3}J_{C2'H1}$ should be possible in ^{13}C-edited 2D NOESY or 2D ROESY spectra of oligosaccharides containing levels of ^{13}C such that the population of the molecules with ^{13}C in two adjacent residues is negligible. This report describes the measurement of the inter-glycosidic, long-range ^{13}C–^{1}H scalar coupling constants in the cyclic β(1→2)-glucan icosamer via ^{13}C-half-filtered {^{1}H,^{1}H} dipolar correlated spectroscopy, and the complementary measurement of the intra-residue long-range ^{13}C–^{1}H scalar coupling constants via ^{13}C-half-filtered {^{1}H,^{1}H} scalar correlated spectroscopy.

MATERIALS AND METHODS

A size-heterogeneous preparation of cyclic β(1→2)-glucans, isolated by anion-exchange chromatography of the culture fluid of Exo-strain ANU 437 (Chakrovorty et al., 1982) of the bacterium *Rhizobium trifolii*, was kindly provided by Dr. Russell W. Carlson (Complex Carbohydrate Research Center, The University of Georgia, Athens, GA). Size-homogeneous cyclic β(1→2)-glucans were isolated from this mixture by reverse-phase HPLC (Koizumi et al., 1983) on a Hibar Lichrosorb RP-18 octadecyl silica column (250 × 10 mm) eluted with 4% aqueous CH_3OH at a flow rate of 3 ml/min. The cyclic icosamer (i.e., the species containing 20 glucosyl residues, see Fig. 1) was used for the NMR spectroscopic studies.

NMR spectra of 5 mM solutions of the glucan icosamer in $D_2O/(CD_3)_2CO$ (9:1, v/v) were recorded with a Bruker AMX-600 spectrometer at 32 °C. The pulse sequence used for the {^{1}H,^{1}H} ROESY experiment with a ^{13}C-ω_1-half-filter is depicted in Fig. 2a. The acquisition times in the t_1 and t_2 domains were 0.106 s and 1.704 s, respectively, with Δ_1 = 1.1 s, Δ_2 = 1.45 s, δ_1 = 3 ms, and δ_2 = 1 ms. The spin-lock purge pulse (SL_x) was applied for 1.7 ms. The ROESY spin-lock (SL_y) was achieved by a train of short pulses (about 20° each) (Bax, 1988), with a total duration of 155 ms and an effective field strength of 2.7 kHz. The carrier frequency was moved 1 kHz downfield from the center of the spectrum during the ROESY mixing time. For each t_1 increment, 160 scans were accumulated, resulting in about 54 h of total measuring time. The pulse sequence for the {^{1}H,^{1}H} COSY experiment with ^{13}C-ω_1-half-filter is depicted in Fig. 2b. The acquisition parameters were the same as described for the ROESY experiment, except that 32 transients were accumulated for each t_1 increment, resulting in a total measuring time of 11 h. The data were processed on a Silicon Graphics Personal IRIS 4D/220 GTX computer with the FELIX software package, version 2.0 (Hare Research Inc., 1991).

RESULTS AND DISCUSSION

The inter-glycosidic coupling constants $^3J_{C1H2'}$ and $^3J_{C2'H1}$ for the cyclic β(1→2)-glucan icosamer were measured in a ^{13}C-filtered {^1H,^1H} ROESY experiment, while the complementary intra-residue $^2J_{C1H2}$ and $^2J_{C2H1}$ were measured independently in a ^{13}C-filtered {^1H,^1H} COSY spectrum of the compound.

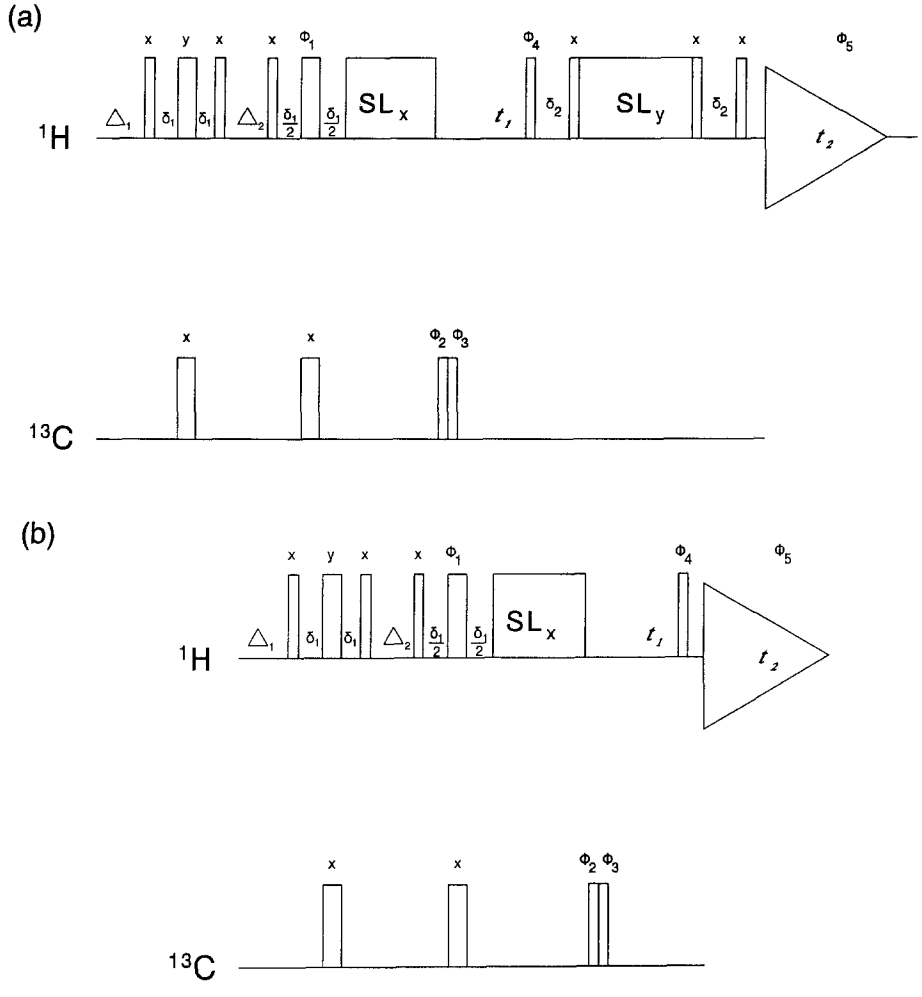


Fig. 2. (a) The pulse sequence used for the ^{13}C-ω_1-half-filtered {^1H,^1H} ROESY experiment. Either 90° or 180° pulses were used (distinguished by their width in the diagram). The SL_y was composed of a train of short, about 20°, pulses separated by short delays (Bax, 1988). The RF carrier was moved away from the center of the spectrum during the ROESY spin-lock period and back during the remainder of the experiment in order to avoid Hartmann–Hahn effects (Rance, 1987). The delay, δ_1, was set to $1/(2^1J_{CH})$ and Δ_2 was adjusted experimentally to minimize the $^1H(-^{12}C)$ signal. The phases of pulses and receiver were cycled as follows: φ_1 = 8(x), 8(−x), φ_2 = 4(x), (−x), 2(x), (−x), φ_3 = (x), 2(−x), 5(x), φ_4 = 16(y), 16(−y), φ_5 = (x), 2(−x), (x), (−x), 2(x), 2(−x), 2(x), (−x), (x), 2(−x), (x). After 16 scans the phase of the receiver was incremented by 180°. TPPI was applied to phase φ_4. (b) The pulse sequence used for the ^{13}C-ω_1-half-filtered {^1H,^1H} COSY experiment. All parameters had the same values as for the ROESY experiment (Fig. 2a), except for the phase φ_4 which was in this case 8(x),8(y).

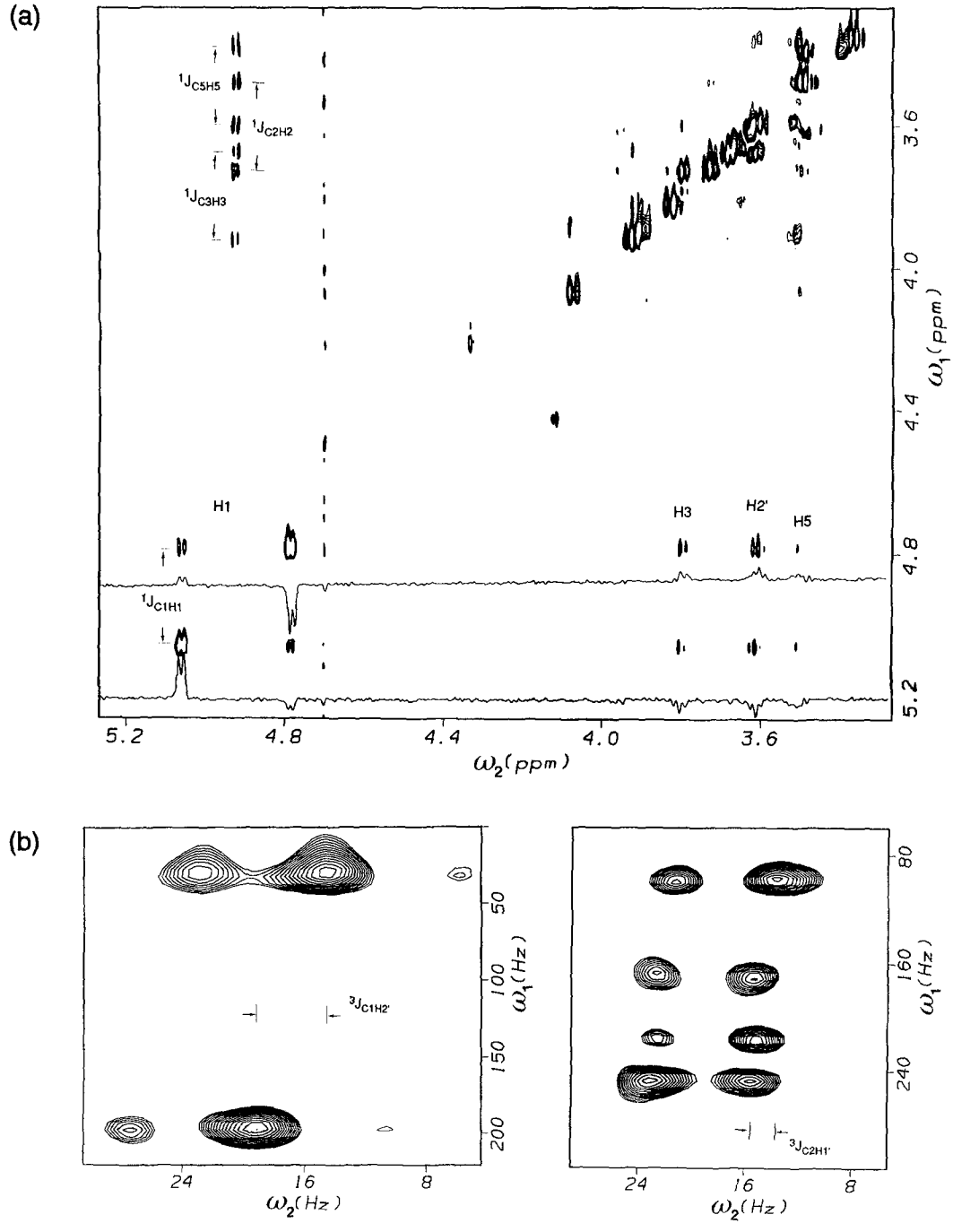
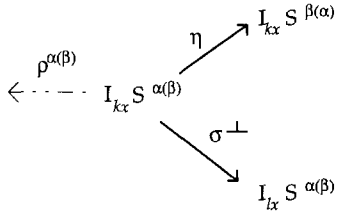


Fig. 3. (a) ^{13}C-ω_1-half-filtered {^{1}H,^{1}H} ROESY spectrum of the cyclic β(1→2)-glucan icosamer. Data were zero-filled in both time domains and multiplied by a Lorentz-Gauss window function before Fourier transformation. The resulting digital resolution was 0.293 Hz/pt and 4.7 Hz/pt in the ω_2 and ω_1 dimensions, respectively. The spectrum is displayed in phase-sensitive mode. Positive and negative levels were not distinguished, except in the traces through the H1 components. (b) Blow-up of the H1/H2′ (left) and H2′/H1 (right) multiplet regions of the spectrum shown in Fig. 3a.

{$^1H,^1H$} ROESY with ^{13}C-ω_1-half-filter

Figure 3a shows the ^{13}C-ω_1-half-filtered {$^1H,^1H$} ROESY spectrum of the cyclic β(1→2)-glucan icosamer obtained with the pulse sequence depicted in Fig. 2a. Because this type of experiment is very sensitive to spectrometer instabilities, we found it necessary to incorporate the $BIRD_y$ pulse (Garbow et al., 1982) during the relaxation delay of the experiment in order to suppress ^{12}C-attached proton magnetization (Otting and Wüthrich, 1990). Unfortunately, this modification resulted in a loss of sensitivity because of negative ^1H–^1H NOE effects (Bax and Subramanian, 1986). Moreover, the signal-to-noise ratio in the spectrum of the glucan recorded with NOESY magnetization transfer was inferior to that obtained by the analogous ROESY experiment. We, therefore, restrict this discussion to the ROESY spectrum (Fig. 3), the appearance of which can be understood as follows.

The ^{13}C half-filter that precedes the t_1 evolution period selects anti-phase proton magnetization with respect to $^1J_{CH}$ coupling. By making use of the product operator formalism of Sørensen et al. (1983), this proton magnetization may be written as: $I_xS_z = I_xS^\alpha - I_xS^\beta$, where the terms to the right correspond to α and β polarization of the carbon spin, respectively. The relaxation pathways for both terms during the spin-lock time are depicted in the following scheme:



Transfer of magnetization along the η and $\sigma^\perp$ pathways gives rise to off-diagonal cross peaks in the 2D spectrum, while ρ is the decay rate of the diagonal signals. It should be mentioned that this decay rate is not necessarily equal for the α and β components (vide infra).

The most pertinent event in the ROESY experiment is the transfer of magnetization from proton k to proton l, governed by the rotating-frame cross-relaxation rate, $\sigma^\perp$ (Bothner-By et al., 1984). Since no carbon pulses are given after the filtering step of the experiment (Fig. 2a), the cross-peak pattern is exclusive, i.e., correlations are observed only between proton signals associated with the same polarization of carbon spin (Griesinger et al., 1985; Montelione et al., 1989). Expansions of the H1/H2′ and H2′/H1 cross peaks are shown in Fig. 3b. The long-range $^3J_{C1H2'}$ and $^3J_{C2'H1}$ coupling constants were measured as displacements in the exclusive-type spectra; they were found to be 4.7 ± 0.3 Hz and 2.2 ± 0.3 Hz, respectively. The sign of these $^3J_{CH}$ couplings is the same as that of $^1J_{CH}$, which we assumed to be positive (compare Poppe and Van Halbeek, 1991a).

The auto-correlation peaks of the anomeric proton (Fig. 3a) are the result of transfer of magnetization between the components of the diagonal multiplets, caused by ^{13}C–^1H dipolar interaction. The transfer rate, η, depends upon the spectral density at the carbon frequency only (Goldman, 1984).

If longitudinal relaxation occurs predominantly through the dipole–dipole and chemical shift

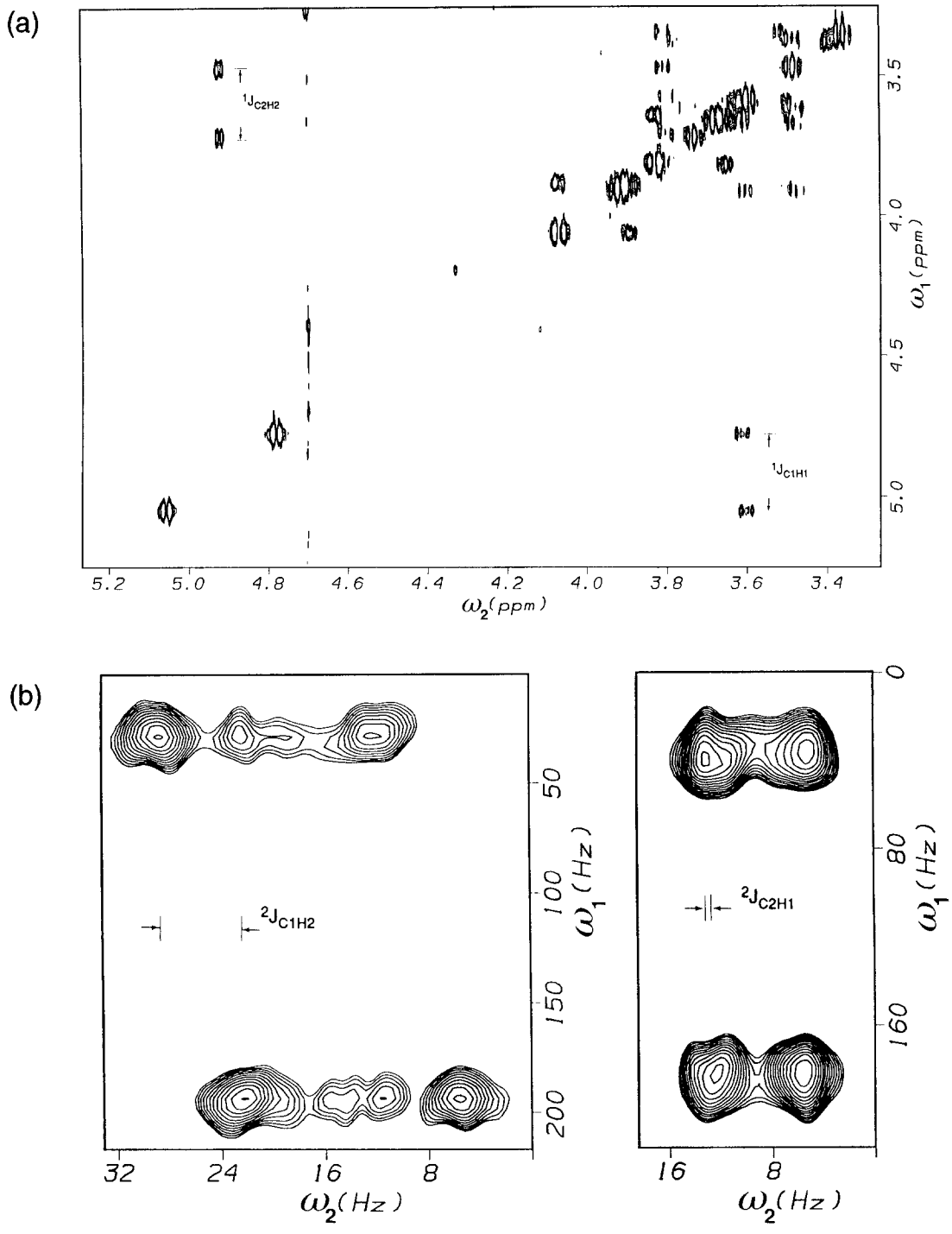


Fig. 4. (a) ^{13}C-ω_1-half-filtered {^1H,^1H} COSY spectrum of the cyclic β(1→2)-glucan icosamer. The spectrum is displayed in magnitude mode. (b) Expansion of the H1/H2 (left) and H2/H1 (right) multiplet regions of the spectrum shown in Fig. 4a.

anisotropy mechanisms, the decay rates ρ of the I_xS^{α} and I_xS^{β} magnetizations should differ due to cross-correlated dipolar and chemical shift anisotropy interactions, as described theoretically for the ortho-ROESY experiment (Goldman, 1984; Brüschweiler and Ernst, 1992). Indeed, the intensities of the anomeric proton signal components on the diagonal in the spectrum shown in Fig. 3 differed by about 20%. We ascribe this difference in intensity to cross-relaxation caused by cross-correlation between chemical shift anisotropy of the anomeric proton and the ^{13}C-^{1}H dipolar interaction. We have confirmed this relaxation mechanism independently for [^{13}C1]-β-glucose (Poppe and Van Halbeek, unpublished results).

{$^{1}H,^{1}H$} COSY with ^{13}C-ω_1-half-filter

The intra-ring $^{2}J_{C1H2}$ and $^{2}J_{C2H1}$ coupling constants were measured in a ^{13}C-filtered COSY experiment (Otting and Wüthrich, 1990). The pulse sequence of this experiment was analogous to that described above, but the ROESY magnetization transfer was replaced with a COSY step (see Fig. 2b). Four-bond scalar coupling effects for the interactions between anomeric and aglyconic protons are negligible (i.e., $^{4}J_{H1H2'} = {}^{4}J_{H1'H2} \leqslant 0.1$ Hz); therefore, COSY transfer occurs only between protons within the same residue. The {^{1}H,^{1}H} COSY spectrum of the cyclic glucan icosamer, obtained with ^{13}C-ω_1-half-filter, is shown in Fig. 4a. Expansion of the H1/H2 and H2/H1 multiplets (Fig. 4b) reveals $^{2}J_{C1H2}$ and $^{2}J_{C2H1}$ coupling constants of -6.6 ± 0.3 Hz and -0.3 ± 0.3 Hz, respectively. The sign of these $^{2}J_{CH}$ couplings is opposite to that of the $^{1}J_{CH}$ and $^{3}J_{CH}$ couplings (compare Poppe and Van Halbeek, 1991a). This is evident from the opposite direction of the cross-peak displacements representing the long-range heteronuclear couplings in the COSY spectrum (Fig. 4b) versus the ROESY spectrum (Fig. 3b).

CONCLUSIONS

We have presented a new approach for measuring inter-glycosidic heteronuclear coupling constants in carbohydrates, based on the spatial proximity of the anomeric and aglyconic protons. The method is particularly suitable for molecules that give highly degenerate ^{1}H-NMR spectra where intra-glycosidic heteronuclear scalar coupling would otherwise interfere with the measurement. The method also can be applied successfully in less difficult cases, where the exclusive nature of the cross peaks and the dispersion of signals in a second dimension compensate for the inherent difficulty of measuring coupling constants having the same magnitude as the line width of the signals.

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