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Note

Application of selective 2D–*J* heteronuclear n.m.r. spectroscopy to long-range coupling constant measurements in the carbohydrate field

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The detection and assignment of long-range ^1H–^{13}C coupling constants (across two or three bonds particularly) in carbohydrates may yield valuable information for molecular identification and conformational analysis[1–6]. By classical ^1H-coupled, ^{13}C-n.m.r. spectroscopy, the measurement of such couplings is practically impossible because of overlapping multiplets. Isotopic enrichment[2,5,6] with ^2H or ^{13}C constitutes one alternative, but it is generally expensive, time consuming, or difficult to achieve.

Some new 2D-n.m.r. techniques which allow direct measurement or assignment (or both) of long range ^{13}C–^1H coupling constants in natural abundance compounds have been recently described[7–13]. In the sequence proposed by Bax and Freeman[7], a selective proton-pulse is used when observing the ^{13}C signal by spin-echoes, so as to affect only the long range ^{13}C–^1H couplings with the selected proton. In order to evaluate the feasibility of this method, ^2J and $^3J_{C,H}$ coupling constants were measured for carbohydrate derivatives and compared with the values already obtained[6,14,15] by other methods for the corresponding ^{13}C- or ^2H-enriched compounds. The following derivatives were used: methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**1**), methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**2**), 1,2,3,4-tetra-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranose (octa-*O*-acetyl-β-gentiobiose) (**3**), and methyl 4-*O*-α-D-glucopyranosyl-β-D-glucopyranoside (methyl β-maltoside) (**4**).

From the values reported in Table I, one can observe that the agreement is generally good. The only noticeable discrepancies are due either to differences found between calculated and measured values ($^2J_{C\text{-}1,H\text{-}2}$ for **1**) or to a lack of resolution for the small coupling constants. The coupling constant $^4J_{C\text{-}1,H\text{-}4}$ of **1** which was 0.5 Hz as measured in the ^1H-n.m.r. spectrum of the ^{13}C-labeled com-

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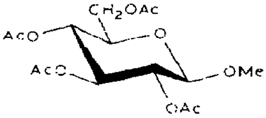

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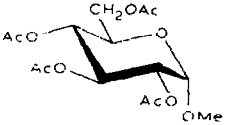

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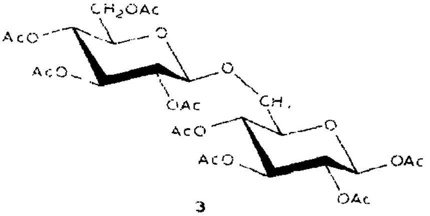

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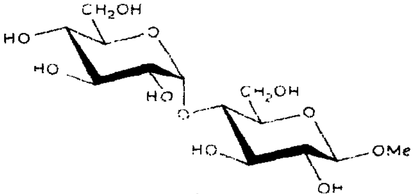

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pound, was below the resolution limit of this study (line width: about 0.6 Hz, as shown in Fig. 1). In this case, the relatively short relaxation-time T_2 (~0.3 s) of C-1 contributed to reduce the echo and broaden this ^{13}C signal. Thus, this technique suffers from limitation of the solution of carbon signals with short T_2. This could be particularly crucial in the case of oligo- or poly-saccharides for which the T_2 values fall <~0.2 s.

Nevertheless, this spin-echo 2D method permits, for natural abundance compounds with appropriate T_2 values, an easy observation of all long range ^{13}C–^{1}H coupling constants for any isolated proton (at least 40 Hz away) in the ^{1}H-n.m.r. spectrum. Care should be taken when protons are strongly coupled or when proton satellites of ^{13}C atoms are overlapping the irradiated region (thus leading to "Selective Population Transfer" from unexpected carbon atoms). The use of high-field spectrometers should partly overcome these difficulties.

This study showed that a good agreement was obtained between the coupling constants previously reported with labeled molecules and the values directly measured by the sequence of Bax and Freeman[7] using natural abundance compounds. With this technique, spectroscopists are able to measure precisely long-range, heteronuclear spin–spin coupling constants for many compounds. A better understanding of $^{2}J_{C,H}$ couplings would result. Moreover, all the collected data will help to establish well-fitted Karplus-type relationship between $^{3}J_{C,H}$ measurements and the corresponding dihedral angles. This could be particularly useful for the conformational study of oligosaccharides by $^{3}J_{C,H}$ measurements through the glycosidic linkage.

TABLE I

LONG RANGE ^{13}C–^{1}H COUPLING CONSTANTS OF SOME CARBOHYDRATE DERIVATIVES

Compound	*Coupling constants*	*Value (Hz)*		*Ref.*
		Observed[a]	*Reported*[b]	
1	$^{2}J_{C-1,H-2}$	5.4	6.5	12
	$^{3}J_{C-1,H-3}$	0.9	0.7	12
	$^{4}J_{C-1,H-4}$	<0.6	0.5	12
	$^{3}J_{C-1,H-5}$	2.8	2.5	12
	$^{3}J_{C-1,H(OMe)}$	4.8	4.6	12
2	$^{2}J_{C-1,H-2}$	1.2	1.2	12
	$^{3}J_{C-1,H-3}$	0.8	0.5	12
	$^{3}J_{C-1,H-5}$	1.9	2.0	12
	$^{3}J_{C-1,H(OMe)}$	4.7	4.5	12
3	$^{3}J_{C-6,H-1'}$	3.4	3.8	6
	$^{3}J_{C-1',H-6S}$	2.6	2.0	6
	$^{3}J_{C-1',H-6R}$	3.9	4.0	6
4	$^{3}J_{C-4,H-1'}$	4.3	4.0	13

[a]This work. [b]In ref.

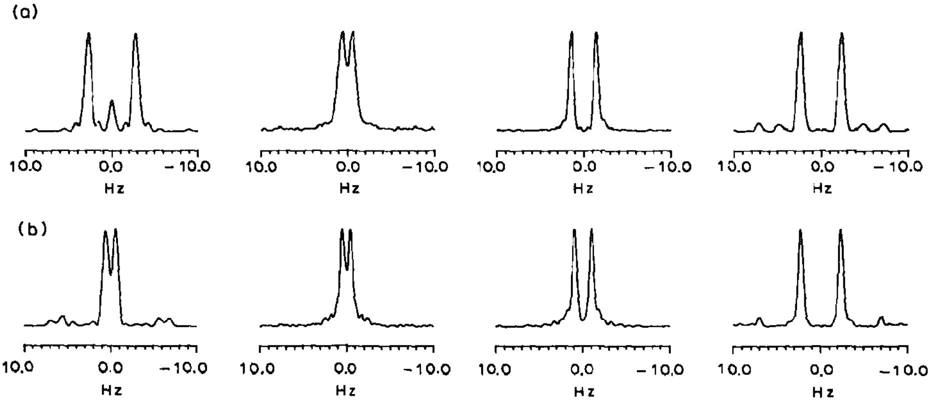

Fig. 1. Long-range C-1,H couplings obtained from 2D slices corresponding to selective irradiations of H-2, H-3, H-5, and H(OMe), from left to right, respectively, for: (a) compound **1**; (b) compound **2**.

EXPERIMENTAL

Preparation of compounds. — Methyl 2,3,4,6-tetra-*O*-acetyl-β- (**1**) and -α-D-glucopyranoside (**2**) were obtained according to classical methods. β-D-Gentiobiose octaacetate (**3**) was prepared in our laboratory but is now available commercially. Methyl β-D-maltoside (**4**) was also prepared in our laboratory from maltose by use of the well-known Koenigs–Knorr reaction.

N.m.r. spectroscopy. — All samples were dissolved either in ($^2H_6$)acetone (compound **1**), ($^2H_6$)benzene (**2**), (^{2}H)chloroform (**3**), or D_2O (**4**) as 25–40 mg/mL solutions. The tubes were sealed after four freeze–pump–thaw cycles. The spectra were recorded with high-field Bruker spectrometers (AM 300 and AM 400). The detection of long-range ^1H–^{13}C spin-coupling constants was achieved according to the selective 2D–*J* heteronuclear sequence proposed by Bax and Freeman[7]. The selective 180° proton pulse was obtained by use of a Dante sequence[16]; 42 pulses were sufficient for selectivity. The evolution period was varied up to 0.5–0.75 s, giving a spectral width of ±10 Hz. The 2D data were processed in the F_2 dimension and only slices corresponding to ^{13}C frequencies were Fourier-transformed in the F_1 dimension. Coupling constants were measured before data manipulations. The best experimental conditions were found for 25 mg/mL degazed solutions (higher concentrations induced line broadening due to shorter T_2).

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