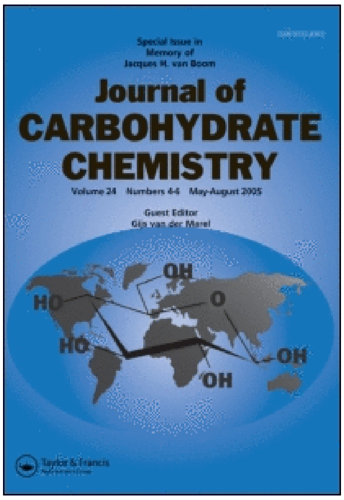

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Hricovíni, M. , Tvaroška, I. , Uhrin, U. and Batta, Gy.(1989) 'Determination of Long Range Proton Carbon Coupling Constants by Modified Semi-Selective Two-Dimensional Inept: An Application in Stereochemical Analysis of Saccharides', Journal of Carbohydrate Chemistry, 8: 3, 389 — 394

To link to this Article: DOI: 10.1080/07328308908048568

URL: http://dx.doi.org/10.1080/07328308908048568

J. CARBOHYDRATE CHEMISTRY, 8(3), 389-394 (1989)

DETERMINATION OF LONG RANGE PROTON CARBON COUPLING CONSTANTS BY MODIFIED SEMI-SELECTIVE TWO-DIMENSIONAL INEPT: AN APPLICATION IN STEREOCHEMICAL ANALYSIS OF SACCHARIDES

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Received July 6, 1988 - Final Form November 21, 1988

Long-range proton-carbon coupling constants are useful in the assignment of ^{13}C NMR spectra and in stereochemical analysis. The measurement of vicinal coupling constants, $^{3}J_{C-H}$, and their interpretation based on appropriate Karplus-type relationships (e.g. for $^{3}J_{H-C-C-C}$ or $^{3}J_{H-C-O-C}$)[1-3] provide valuable information in conformational studies of carbohydrates. Nevertheless, the use of $^{3}J_{C-H}$ in carbohydrate studies is rather rare because their measurement is time consuming and analysis of ^{1}H-coupled ^{13}C NMR spectra is complicated. However, 2D NMR methods[4-6] that allow precise measurement of long-range couplings in a reasonable time have become available recently.

In 2D J-spectroscopy Bax and Freeman[4] proposed a semi-selective-nonselective combination of 180° ^{1}H and ^{13}C pulses. By using a systematic variation of the evolution period, the doublets (multiplets) due to long-range coupling can be observed since only the chosen proton (protons) modulates the ^{13}C spin echo after the application of the soft ^{1}H pulse. In the preparation period the nuclear Overhauser enhancement was used to increase the spectral signal-to-noise ratio.

Higher sensitivity is achieved in the semi-selective 2D INEPT experiment[5] in which the effect of polarization transfer is utilized. Nonselective ^{1}H 90^o pulses are applied on the spin system in the pulse sequence. After the interval $t_1/2$ (in the midpoint between ^{1}H 90^o pulses), the applied semi-selective 180^o pulse affects only magnetization of the preselected proton. Therefore, only the ^{13}C magnetization of carbons coupled via long-range couplings to preselected protons is present in the second half of the pulse sequence. Since ^{13}C doublets arising from long-range couplings are detected exclusively, the resulting 2D spectrum is simple. However, because the ^{1}H 90^o pulses are nonselective, the power of the decoupler must be altered (high and low range) four times during experiment. This can be a rather demanding task for decoupler hardware in some types of spectrometers.

A modified 2D heteronuclear shift-correlated experiment[6] is an alternative approach for measuring long-range couplings. In a single experiment C-H couplings can be determined over one on more bonds, although some signals may not be observed due to inappropriate setting of timing parameters in the pulse sequence. This method requires a large computer memory, and the sensitivity of the spectrometer must be high.

Until now, the 2D semi-selective J-resolved experiment has been used exclusively in the measurement of long-range couplings of saccharides[7-9].

In this paper, a modified version of 2D semi-selective INEPT for the determination of three-bond heteronuclear coupling constants ($^3J_{H-C-O-C}$) is presented in which both nonselective 90^o proton pulses are replaced with soft pulses. This modification reduces considerably the number of changes of decoupler power levels during the experiment (FIG. 1). The potency of the method is demonstrated on 1,6:2,3-di-anhydro-D- gulopyranose (1) and methyl-4-O-β-D-xylopyranosyl--β-D-xylopyranoside (2) which represent both rigid and flexible saccharides in solution, respectively.

Experiments were performed on a Bruker AM-300 spectrometer with Aspect 3000 computer. Data matrices were 1K X

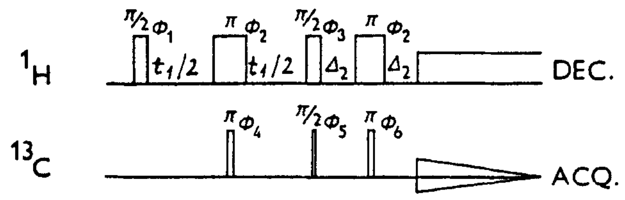

FIG. 1. Pulse sequence of the modified semi-selective 2D INEPT in which all ^1H pulses are soft (10 ms for 90° pulse). The interval, $t_1/2$, varies systematically (typically in 64 steps), and Δ_2 is chosen based on the expected couplings ($\Delta_2 + \tau_{90} = 0.25/J_{C-H}$). The ^1H frequency is set at the position of the irradiated proton.

64 data points, and the spectral width in the F1 dimension was ± 20 Hz. Selected traces were zero-filled to give a final resolution of 0.15 Hz. Prior to performing Fourier transformations, data were multiplied with appropriate weighting functions (shifted sine-bell in the F_2 dimension, Gaussian multiplication in F_1 dimension).

In FIG. 2 is shown a 2D spectrum, and cross sections thereof,(compound 1) using the proposed pulse sequence. By polarization transfer from the H-1 proton, four long-range couplings were observed: $^2J_{C2,H1} = 4.7$ Hz, $^3J_{C3,H1} = 4.1$ Hz, $^3J_{C6,H1} = 6.3$ Hz, $^3J_{C5,H1} = 5.0$ Hz. The ^3J values correspond to the following torsion angles[2]: -150.9°, 147,7°, -163.8°. A compromise setting of the refocusing interval, Δ_2, was used ($\Delta_2 = 45$ ms, which is optimal for 5 Hz if relaxation effects are neglected).

The glycosidic linkage of a disaccharide in solution has a pair of flexible H-C-O-C bonds. Since the NMR time scale is slower than that for the reorientation of molecules in solution, the observed values define an averaged conformation. The NMR derived structure represents an average of several conformers. The extent of the averaging can be assessed from the temperature dependence of the coupling constants, and theoretical calculations[10].

The $^3J_{H-C-O-C}$ coupling constants at different temperatures for compound (2) in ^2H_2O are listed in the Table 1. H-1'

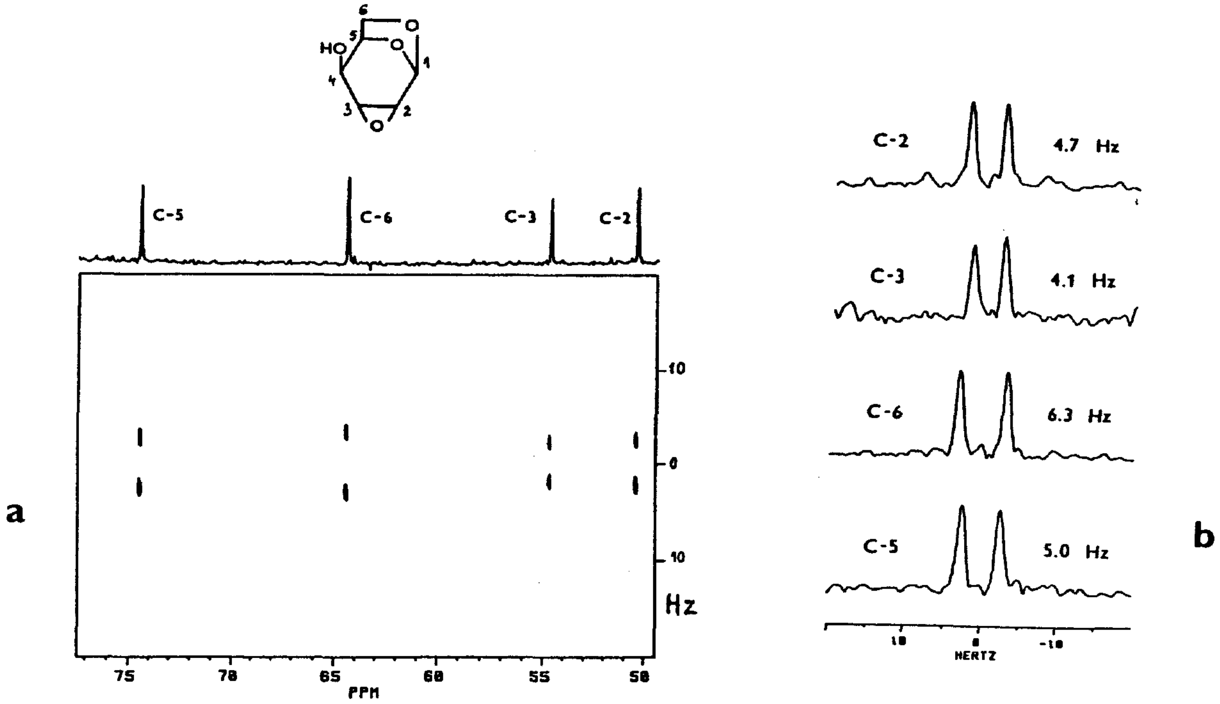

FIG. 2. 2D J-spectrum (a) and cross-sections (b) of (1) in D_2O at 300 K obtained using the pulse sequence in Fig. 1. H-1 was irradiated by a semi-selective pulse (10 ms for 90^0 flip angle). The delay, Δ_2, was set at 45 ms, which corresponds to a J = 5 Hz.

TABLE 1. Three-bond $^{13}C-^{1}H$ interresidue coupling constants, J^{Φ} and J^{Ψ}, measured at different temperatures.

T(K)	278	298	318	338	358
J^{Φ} (Hz)	4.8	4.7	4.4	4.3	4.1
J^{Ψ} (Hz)	5.6	5.1	4.7	4.4	4.2

and H-4 were irradiated separately in two different experiments, giving two different coupling constants across the glycosidic linkage: J^{Φ} for the H-1'-C-1'-O-1-C-4 coupling pathway, and J^{Ψ} for the H-4-C-4-O-1-C-1' pathway.

It can be seen that the inter-residue coupling constants change with temperature.
Differences between J^{Φ} and J^{Ψ} reflect the fact that rotations around tha C-1'- O-1' and O-1'- C-4 bonds are not equivalent[11], the former influenced by the well-known exo-anomeric effect[12]. A detailed interpretation of these data using theoretical conformational calculations to provide the geometries and populations of individual conformers will be presented elsewhere[10].

The modified 2D semi-selective INEPT experiment described in this report is a convenient technique for stereochemical investigations because it exhibits high sensitivity and is easy to interpret(no singlet resonances). In comparison with the semi-selective J-resolved experiment, approximately half of the number of transients were sufficient to achieve the same signal-to-noise ratio in our experiments. Nevertheless, problems can arise when the T_2 relaxation times of carbons are short ($<$0.2 s) and/or in selecting a compromise setting of the refocusing period when a large range of coupling constants is expected.

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