

Proton NMR Spectra of Galactitol

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Introduction

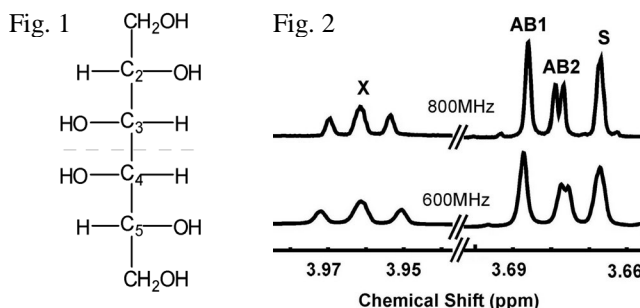
Galactitol has been known to elevate in the urine of patients with galactosemia caused by galactose-1-phosphate uridylyltransferase deficiency, even under a galactose-restricted diet [1]. It has been hypothesized that elevated galactitol levels may be present in the brain of these patients [2]. The ability to detect low levels of galactitol by *in vivo* ¹H MR spectroscopy may have a clinical value for managing these patients. The galactitol peaks are present from 3.6 to 4.0 ppm, and overlap with myoinositol, glutamine and glutamate. Therefore, there is a need for spin-editing methods. However, the coupling pattern between various protons in galactitol has not been elucidated in the literature. The purpose of this work is to measure the chemical shifts and coupling constants among various protons in galactitol.

Methods

The chemical structure of galactitol is shown in Fig 1. The molecule is symmetric about the horizontal dashed line in the figure. Various NMR measurements of galactitol solution were performed at different field strengths and analyzed together with published data [3,4] (Table 1). Chemical shifts of non-exchange protons and J-coupling constants were obtained analytically [5] based data at 800 MHz. A computer program was written in IDL to calculate the line shapes in a system with eight coupled 1/2 spins to confirm the results using matrix operator formalism.

Table 1. Experimental data

Proton Frequency (MHz)	Solvent for galactitol solution	Measurement
64	H ₂ O	STEAM [3]
400	D ₂ O+DMSO	FID, homonuclear decoupling
400	D ₂ O	FID [4]
600	D ₂ O	FID, COSY
800	D ₂ O	FID



Results

The lower trace of Fig. 2 shows the spectrum obtained at 600 MHz using TSP as a chemical shift reference. The upper trace is the spectrum obtained at 800 MHz. The area integrals of the major peaks from the 800 MHz spectrum (TR = 10sec, flip angle = 10°) had a ratio X(triplet):AB1:AB2(doublet):S = 1:0.99:0.94:1.01. The triplet at 3.962 ppm (X in Fig. 2) is assigned to C2 (or C5) protons. The splitting of the 3.962 ppm triplet is caused by the coupling to the two protons on C1 (or C6). The peaks at 3.67 to 3.69 ppm (AB1 and AB2) are assigned to protons on C1 (or C6). The 3.667 ppm peak (S) is assigned to the protons on C3 (or C4). The spectra are consistent with that of an ABX system [5] and a single uncoupled peak. This finding is consistent with all data we have from 63 MHz to 800 MHz. The results of chemical shifts and coupling constants are summarized in Table 2. Computer simulations ruled out alternative assignments. Surprisingly, there is no coupling between the protons bonded to C2 and C3 (C5 and C4). Because of the lack of coupling between these protons, the C3 and C4 protons become magnetically equivalent and only one single line is observed. These assignments were supported by a 2D COSY measurement at 600 MHz, which showed little cross peak intensity between Peak S and other peaks. In a DMSO solution containing 20% of D₂O at 400 MHz, the spectral pattern consisted again of an ABX and a single peak. When homonuclear decoupling was performed by irradiating the X spin, the single peak was not affected.

Discussion

Measurement of chemical shifts and couplings in galactitol is important for the design of pulse sequences for optimal measurement of this metabolite *in vivo*. The chemical shifts in Table 2 are consistent with previous values at 400 MHz [4]. However, the chemical shifts of the two C1 protons were not resolved previously [4], and the coupling constants were not reported. In most molecules, protons bonded to neighboring carbons are coupled with a coupling constant of approximately 7 Hz. It is very unusual that this coupling is not observed between the C2 and C3 (C5 and C4) protons. This observation probably indicates that rotation around the C2-C3 bond is restricted and the molecule adopts a conformation in which the dihedral angle H-C2-C3-H is close to 90 degrees. This feature leads to a significant simplification of the spectrum. If we neglect the coupling between protons across 4 chemical bonds, galactitol can be treated as an ABX system plus one uncoupled peak at high fields. At 1.5 T, the chemical shift difference between the AB protons will not be important. However, the difference between J_{AX} and J_{BX} may still have a practical impact, and the C1 and C2 protons are neither weakly nor strongly coupled.

Table 2. Results for galactitol in D₂O

Proton	Chemical Shift (ppm)	J-coupling
C1_1	δ _A = 3.680	C1_1 and C2: J _{AX} = 7.9 Hz C1_1 and C1_2: J _{AB} = -11.5 Hz
C1_2	δ _B = 3.685	C1_2 and C2: J _{BX} = 4.9 Hz
C2	δ _X = 3.962	C2 and C3: 0 Hz
C3	δ _S = 3.667	

References

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