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Note

Determination of the activation energy and rate constants for the chair-to-chair interconversion of *cis*-inositol by n.m.r. spectroscopy

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The solution dynamics of carbohydrates and their chelation of metal ions have been of interest to scientists for a number of years[1,2]. Solution dynamics may affect the structures of complex oligosaccharides (*e.g.*, antigen epitopes) and carbohydrate-metal ion interactions may influence events occurring at cell surfaces[3,4].

As a beginning to the investigation of carbohydrate solution dynamics as well as interactions with metal ions we decided to focus on a simple carbohydrate analogue. The model system we chose is *cis*-inositol (**1**). In this symmetrical compound hydroxyl groups can be found in alternate axial and equatorial arrangements, and the two chair forms are energetically equivalent. Furthermore, its axial-equatorial-axial "trihydroxyl" pockets provide chelation sites for metals having certain ionic radii[1].

Using n.m.r. line-shape analysis we obtained rate constants for the chair-to-chair interconversion of *cis*-inositol as well as the activation parameters for this process. The effect of Ca^{2+} on the interconversion of **1** was also investigated.

EXPERIMENTAL

1,2:3,4-Di-*O*-cyclohexylidene-*cis*-inositol was a gift from Dr. Stephen J. Angyal of the University of New South Wales, Australia. As previously described, it was converted into *cis*-inositol and the ^{13}C-n.m.r. spectra were determined[5]. For the present study, ^{1}H-n.m.r. spectra were recorded in D_2O on an IBM AE200 n.m.r. spectrometer operating at 200 MHz. The chemical exchange rate constants for the chair-to-chair interconversion were obtained by comparing experimental spectra and the simulated spectra.

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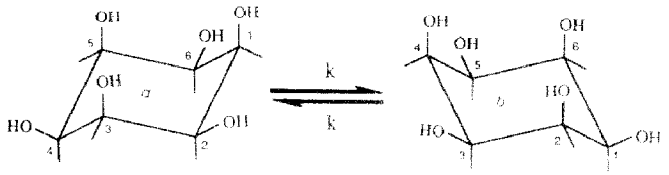

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The chair-to-chair interconversion of *cis*-inositol may be described as a two-site exchange problem of spin multiplets, *i.e.*,

$$A_2X_2 \overset{1/\tau}{\rightleftharpoons} A_2X_2$$

where τ is defined as the lifetime of the chair form[6]. We can obtain the chemical exchange rate constant (k) for the interconversion by the relationship

$$k = \tau^{-1}$$

N.m.r. line-shapes arising from a two-site chemical exchange between simple resonances (*i.e.*, the spectrum of the spins in each site is a single, nonoverlapping Lorentzian line without multiplicity) are well known[7]. However, the analysis is applicable to more complicated exchanges involving coupled spin systems[8,9]. For the *cis*-inositol system, the exchange leads to a signal intensity at the frequency ω given by

$$\psi(\omega) = [A]\,\mathrm{Im}(\chi_A) + [X]\,\mathrm{Im}(\chi_X),$$

in which $[A]$ and $[X]$ are the concentrations of axial and equatorial protons, respectively. Im represents the imaginary part, and χ_A and χ_X are the functions of ω which can be obtained by solving the matrix equation

$$(i\omega\mathbf{1} + \mathbf{E})\chi = i\mathbf{W}.$$

In this equation **1** is a unit matrix, **E** is a complex matrix independent of ω representing the exchange, and **W** is a vector describing the weighting factors for the spin system. For this specific spin system, **E** consists of a set of three 2×2 matrix equations containing resonance frequencies, resonance linewidths, spin coupling constants, and the interconversion rate constant; χ and **W** are column vectors.

A simulation program for our data, based on this theory, was written in microsoft-BASIC and designed for use with Macintosh or IBM personal computers. Simulated spectra can be obtained by entering resonance frequencies, linewidths of the resonances, and the chemical exchange rate constant.

The temperature of the sample was calibrated using 4% methanol-d_4 in methanol (at temperatures below 300 K) and 80% ethylene glycol in Me_2SO-d_6 (at temperatures above 300 K). Temperature readings were taken before and after each spectral accumulation and the error in the value of the temperature was found to ≤ 0.5 .

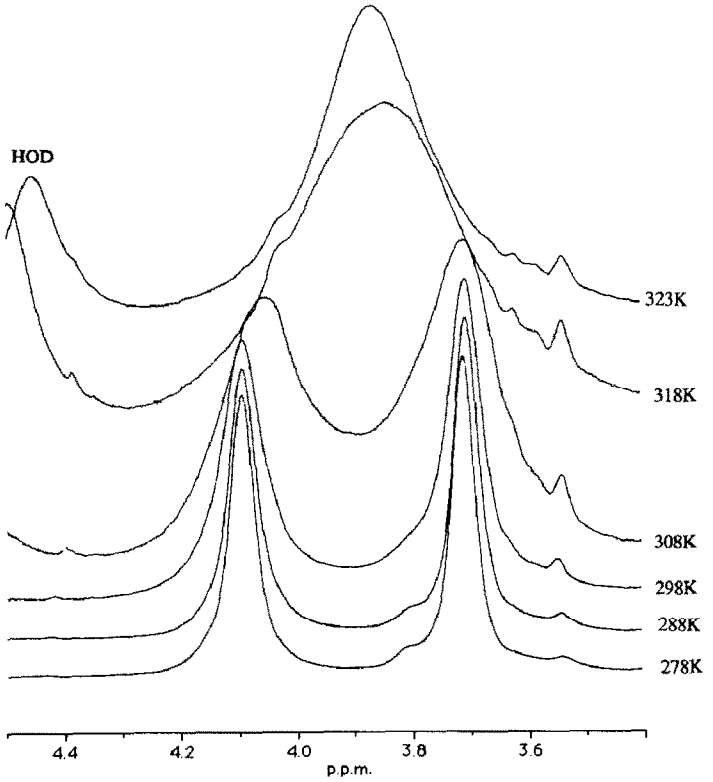

Fig. 1. ^{1}H-N.m.r. spectra of **I** (1.0M, pH 7.0) in D_2O at various temperatures.

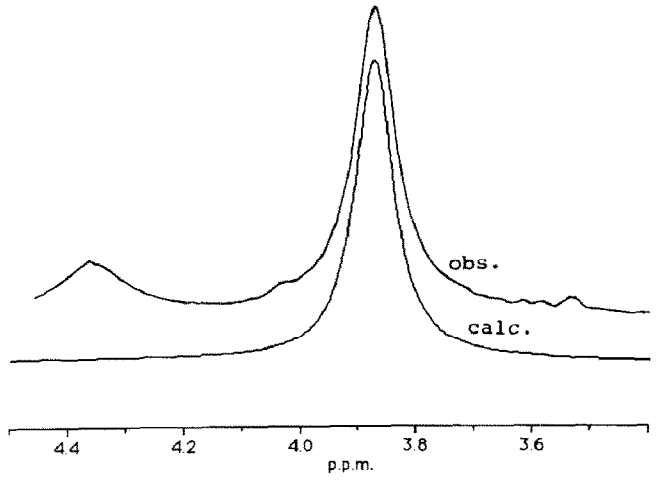

Fig. 2. Comparison of the experimental (obs) and simulated (calc) spectra of *cis*-inositol at 333 K. The parameters are the same as those for the spectra of Figs. 1 and 3.

RESULTS AND DISCUSSION

^{1}H-N.m.r. spectra of *cis*-inositol at various temperatures are given in Figs. 1 and 2. At low temperatures there are clearly two resonances present in the spectra; these represent protons in the axial and equatorial positions. As the temperature is increased, the resonances eventually coalesce, indicating an increase in the exchange rate. Using the equations given in the experimental section, simulated spectra for this exchange process were obtained as shown in Figs. 2 and 3. Note how closely the spectra in Figs. 1 and 2 match those provided in Fig. 2 and 3, indicating excellent agreement between experiment and theory. In the actual ^{1}H-n.m.r. spectra of *cis*-inositol there is an additional resonance (HDO), and its chemical shift is temperature-dependent.

Each temperature point provides data on the kinetics of the exchange process. The rate constants are given in Table I. As expected, the rate of interconversion goes up as the temperature rises, with the rate constant increasing from 7 s^{-1} at 278 to 300 s^{-1} at 333 K. An Arrhenius plot of the data (Fig. 4) gives a value of 53.0 ($\pm$ 1.9) kJ mol^{-1} for the activation energy (E_a) of interconversion. This value is almost double the value previously obtained for the chair-to-chair interconversion of several dimethylcyclohexyl systems[10]. Obviously the difference must be due to the bulky hydroxyl groups.

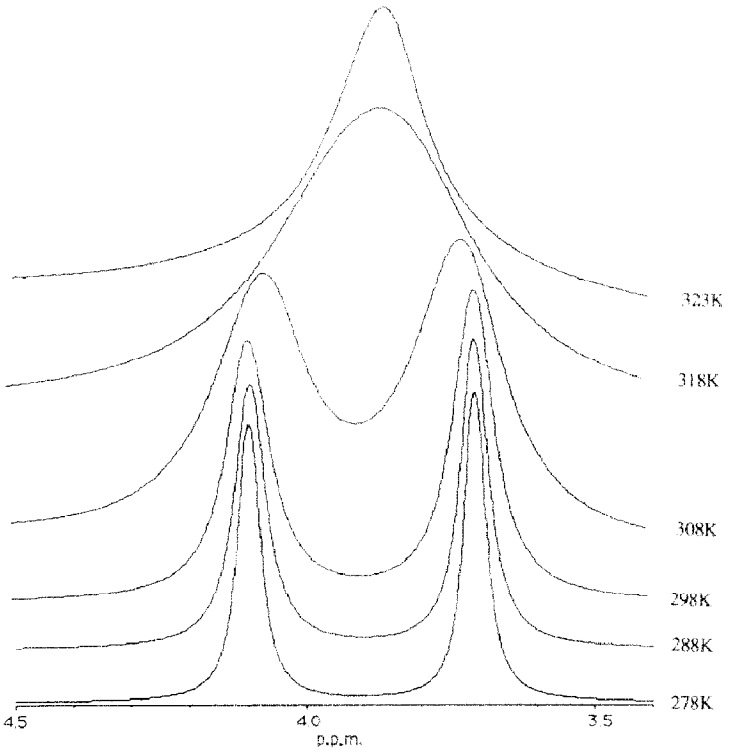

Fig. 3. Simulated spectra for *cis*-inositol. The parameters used were ω_A = 4.10 p.p.m., ω_X = 3.72 p.p.m., J_{AX} = 2.8 Hz, $\Delta\omega_A$ = $\Delta\omega_X$ = 2.2 Hz

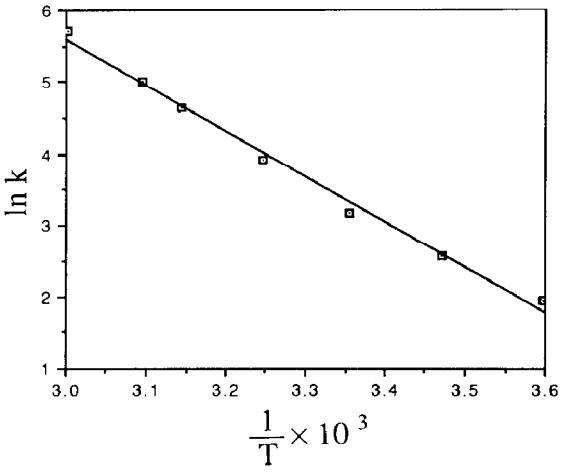

Fig. 4. Plot of ln k as a function of the inverse of the absolute temperature.

TABLE I

Values of the interconversion rate constant for *cis*-inositol as a function of temperature

T (*K*)	k(s^{-1})[a]	
	cis-*Inositol*	cis-*Inositol plus* Ca^{2+}, *1:1*
278	7	1[b]
288	13	3[b]
298	24	9
308	55	26
318	105	55
323	150	80
333	300	150

[a] Errors in the values are ≤ 10%. [b] Value obtained by the selective inversion recovery method (see ref. 11 for details).

On the basis of previous results, it was expected that Ca^{2+}, having an ionic radius of ~1 Å (ref. 1), would interact preferentially with one of the axial-equatorial-axial hydroxyl sequences in the molecule[1,2], *e.g.*, O-1–O-2–O-3 in conformer *a*. This interaction would slow down the interconversion of the two chair forms. Table I shows the effects of added Ca^{2+} (at a *cis*-inositol:Ca^{2+} ratio of 1:1) on the rate constants of the interconversion. The value is decreased by almost an order of magnitude at 278 K but only by a factor of two at the highest temperature studied. Therefore, the interaction between *cis*-inositol and Ca^{2+} is weak, and the exchange is fast on the n.m.r. time scale.

In conclusion, our results show that the chair-to-chair interconversion of *cis*-inositol does occur slowly at room temperature. Furthermore, the interaction of Ca^{2+} with *cis*-inositol slows down this interconversion.

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