Analysis of the Proton, Carbon, and Nitrogen NMR Spectra of *cis*,*cis*- and *cis*,*trans*-1,3,5-Triaminocyclohexane

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The proton spectrum of the isomeric mixture of cis,cis- and cis,trans-1,3,5-triaminocyclohexane was assigned with a 2D COSY experiment. Using Forrest's modified equation and the 2D J spectrum results, the conformations of the two isomers were analysed. The six-membered ring of the cis,trans isomer is flattened by 1,3-diaxial interactions between two amino substituents; in the cis,cis isomer, ring pucker is increased owing to non-bonded interaction between the equatorial amino groups and adjacent methylene protons. The ¹³C and ¹⁵N spin-lattice relaxation of the two isomeric 1,3,5-triaminocyclohexanes was also measured at different pH values; NH_x groups rotate faster with decreasing pH. Rotation/jump rates and barriers are reported, determined from the NT_1^{DD} ratios between ¹⁵N and ¹³C nuclei.

INTRODUCTION

1,3,5-Triaminocyclohexane has attracted interest from inorganic chemists for at least the past 10 years in studies of entropy,¹ stability constants,² heats of formation³ and evaluations of the bonding energy and structure in x-ray studies⁴ when it is chelated to metals of different kinds. In this paper we explore two aspects of 1,3,5-triaminocyclohexane: its conformation and molecular dynamics.

In the historical development of the conformational analysis of cyclic compounds, tools such as x-ray, neutron and electron diffraction, microwave, IR and NMR spectroscopy have been applied. NMR is a popular tool in conformational analysis for the organic chemist because it gives information for solutions, where most reactions occur.

As a result of valence bond calculations, Karplus⁵ obtained theoretical values for the dependence of three-bond proton spin-spin coupling constants on the dihedral angle, ϕ , which could be approximated by the equation

$${}^{3}J(\text{HCCH}) = A\cos^{2}\theta + B\cos\theta + C \qquad (1)$$

This equation forms the basis of an extensively applied method relating vicinal coupling constants to molecular structure. It has also been applied to other three-bond coupling systems such as ${}^{3}J(\text{HNCH})$,⁶ ${}^{3}J({}^{31}\text{PCCH})^{7}$ and ${}^{3}J({}^{199}\text{HgCCH})$.⁸ The utility of Eqn (1) depends on the accuracy of the constants *A*, *B* and *C*, which may vary considerably from compound to compound (depending on bond length, substituents and orientation⁹). Consequently, difficulties arise in the selection of appropriate values for these constants.

Alteration of the constants A, B and C in order to take into account the effect of the electronegativity of

the substituent does not appear to be completely satisfactory, since the effect of an electronegative substituent is also angle dependent.¹⁰

In order to overcome this problem, Forrest¹¹ used Eqn (2) to calculate the *gauche* coupling constant of two protons in disubstituted ethane fragments with a dihedral angle of 60° :

$$J_{\text{calc}} = \left(4.1 + 0.63 \sum_{i=1}^{N} \Delta X\right) \times (1 - 0.462 \Delta X_1)(1 - 0.462 \Delta X_2) \quad (2)$$

where ΔX is the electronegativity difference between the substituent and hydrogen,¹² $\sum_{i=1}^{N} X_i$ is the sum of the ΔX values of all substituents on the ethane framework and ΔX_1 and ΔX_2 are the ΔX values of the two substituents *trans* to the coupled protons. Forrest tested Eqn (2) with 25 cyclohexanes and sixmembered heterocyclic compounds (in which large deviations from 60° are not expected for the *gauche* dihedral angles). The calculations yielded values having an RMS difference of 0.3 Hz from measured values. Applying the Karplus relationship results in the equation

$$J_{\rm calc} = A \cos^2 60^\circ - 0.3 \tag{3}$$

From the calculated value of J [Eqn (2)] and with Eqn (3), a value for A can be determined. This calculated value of A can then be used with the observed coupling constant [Eqn (4)] to calculate a value of the dihedral angle ϕ_{ac} :

$$\cos\phi_{\rm ae} = \sqrt{(J_{\rm obs} + 0.3)/A} \tag{4}$$

With this method, the conformationally restricted compounds *cis,cis*- and *cis,trans*-1,3,5-triamino-cyclohexane have been analysed. Analysis by this method is superior to other methods (e.g. the *R* value method which can be used for $-CH_2CH_2$ -groupings¹³).

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NMR relaxation parameters (T_1 and NOE) can be used to derive important information about solution interactions and molecular dynamics. ¹³C and ¹⁵N relaxation data used together provide a powerful probe of dynamics in organic molecules containing nitrogen. Observation of ¹³C and ¹⁵N is usually accomplished under broad band decoupling, which minimizes the influence of cross-relaxation terms. Hence the ¹³C and ¹⁵N spin–lattice relaxation times depend mainly on the speed and form of reorientational molecular motions.

For ¹³C or ¹⁵N nuclei having directly bonded protons, dipolar interaction with these protons can completely control relaxation, as given for isotropic tumbling within extreme narrowing by the equation

$$1/NT_{1}^{DD} = (\gamma_{H}^{2}\gamma_{X}^{2}h^{2}\tau_{c})/r_{XH}^{6}$$
(5)

where $\gamma_{\rm H}$ and $\gamma_{\rm X}$ are the magnetogyric ratios of ¹H and ¹³C (or ¹⁵N), $r_{\rm XH}$ is the C-H (or N-H) bond distance, N is the number of directly bonded protons and $\tau_{\rm c}$ is the effective correlation time. Even when other mechanisms contribute to the total relaxation rate, $T_1^{\rm DD}$ can be determined if the NOEs are also measured.

In suitable molecules, comparison of ¹³C and ¹⁵N dipolar T_1 values allows the determination of overall tumbling and internal motion of the framework and amino functional groups. This paper reports results for the two isomers of 1,3,5-triaminocyclohexane which contain amino functions in different stereochemical relationships.

EXPERIMENTAL

cis, cis- and cis, trans-1,3,5-triaminocyclohexane were prepared from the trioxime of α -phloroglucinol according to the method of Lions and Martin.¹⁴ The method was modified through the use of redistilled ammonia and cooling of the reaction mixture in a dry-ice-acetone bath during the addition of sodium (Birch reduction). ¹³C spectra were obtained at 90.56 MHz on a Bruker WM-360 WB spectrometer in 10 mm sample tubes, ¹⁵N spectra were obtained at 36.1 MHz on the same spectrometer, using 20 mm sample tubes and ¹H spectra were obtained at 360.1 MHz in 5 mm sample tubes. T_1 data were obtained using the fast inversion recovery technique, with delay times of 2s between inversion recovery sequences. NOE values were determined using gated decoupling with a delay of $10T_1$ between the pulses.

A 1:1 mixture of *cis*, *cis*- and *cis*, *trans*-triaminocyclohexane isomers was generated. The data were acquired from solutions of the mixture (5% in deuteriated chloroform), which ensured that all conditions (temperature, solvent effect, viscosity, pulsing and paramagnetic impurities, if any) were the same for both isomers in each run.¹⁵ The 2D COSY experiment was used to assign the ¹H spectrum and the 2D *J*-resolved experiment was used to measure coupling constants.

The COSY experiment was performed with a $90^{\circ}-t_1-\alpha$ -acquisition pulse sequence.¹⁶ A basic four-

step phase cycle was used to distinguish negative and positive modulation frequencies (hence, quadrature detection in f_1) and suppress unwanted axial peaks.¹⁷ In order to search for cross peaks near the principal diagonal, the mixing pulse (α) was chosen to be 60°, reducing the relative intensity of parallel transitions while still obtaining good sensitivity.¹⁸ The 90° pulse width for the experiment was carefully measured to be 7 μ s. The time t₁ was incremented by 0.91 ms to cover the desired chemical shift range (1100 Hz) in the f_1 dimension; 256 FIDs (2K each) were acquired in ca 1 h. FIDs were zero filled in both dimensions (from 2K to 4K in f_1 and 128 to 256 in f_2) to obtain better digital resolution. Sine bell window functions were applied in both dimensions to remove spurious peaks generated by overlaps of the tails of two diagonal peaks.¹⁹ (Use of the same weighting function along the two axes also leads to a spectrum with exact symmetry about the diagonal²⁰). After the second Fourier transformation, diagonal symmetrization was performed to remove f_1 noise and quadrature imperfections.²⁰

The 2D *J*-resolved spectrum was collected with a $90^{\circ}-t_1-180^{\circ}-t_1$ -acquisition sequence²¹ using 'exorcycle' phase alternation to suppress flip angle errors and spatial inhomogeneities in the radiofrequency field.²² The f_2 spectral width was 1300 Hz over 4K points, giving a digital resolution of 0.63 Hz per point; 256 spectra with t_1 incremented by 12.3 ms gave an f_1 width of 30 Hz with a digital resolution (after zero filling) of 0.16 Hz per point. The total acquisition time was 1 h. In order to obtain a 'proton-decoupled proton' NMR spectrum, a 45° tilt operation was applied before projection along the f_1 axis. Symmetrization along the $f_1=0$ axis was also performed to obtain a clean spectrum.

RESULTS AND DISCUSSION

¹H NMR chemical shifts

The assignment of the ¹H spectrum of cis, cis- and cis,trans-triaminocyclohexane (TACH) (1:1 mixture) is not trivial. Figure 1 shows the results of a COSY experiment (obtained by the method of Bax et al.23) on the triaminocyclohexane mixture in CDCl₃. The diagonal peaks [those falling on or near the principal diagonal $(F_1 = F_2)$] in Fig. 1 arise from magnetization which either maintains the same resonance frequency during t_1 and t_2 (these peaks fall exactly on the diagonal) or is transferred to another resonance in the same spin multiplet. The cross peaks in Fig. 1 are of particular interest because they correlate the shifts of groups of spin-coupled nuclei:23 two resonances at frequencies f_A and f_B connected by a scalar spin-spin coupling have cross peaks at (f_A, f_B) and (f_B, f_A) . The total assignment for these two isomers is shown in Fig. 2 (¹H one-dimensional spectrum). Long-range coupling between e and f is clearly shown in Fig. 1.



Figure 1. Contour plot of the COSY spectrum of the isomeric mixture of triaminocyclohexanes. For acquisition parameters, see text.



Figure 2. Structure and proton assignments for cis, cis and cis, trans isomers (1:1 mixture).

Chemical shifts (relative to TMS) in the two TACH isomers are listed in Table 1. It is not surprising to find that the methylene axial protons (i, h, g, c and b) are more shielded than are the equatorial protons (d, e and f). Moreover, axial proton i in the *cis*, *cis* isomer was anomalously shielded ($\delta = 0.95$ ppm). This shielding is due to the 'sandwich effect' from two

equatorial amino groups.²⁴ A similar result is indicated in the *cis,trans* isomer by comparing protons h and g. Spin h, being sandwiched by the two equatorial amino groups, has a lower chemical shift $(\delta = 0.98 \text{ ppm})$ whereas spin g, having only one equatorial amino group at a vicinal carbon, is less shielded $(\delta = 1.21 \text{ ppm})$.

Table 1. ¹ H chemical and cis,tran ane ^a	shifts for <i>cis,cis-</i> s-triaminocyclohex-
cis,cis	cis,trans
$\delta_{\rm c} = 2.74$	$\delta_a = 3.42$
$\delta_{\rm d} = 1.97$	$\delta_{\rm b} = 3.13$
$\delta_i = 0.95$	$\delta_{ m e}$ = 1.97
	$\delta_{ m f}$ = 1.71
	$\delta_{g} = 1.21$
	$\delta_{\rm h} = 0.98$
^a Concentration, 0.02 м CDCl ₃ ; temperature, 25 are in ppm, relative to 7	(for each isomer) in 5°C. Chemical shifts IMS.

Coupling constants

In order to obtain information about the spin multiplicity, a 2D J-resolved NMR experiment was performed; the experimental method has been described elsewhere.²⁵ Figure 3 shows the contour plot of the 2D ¹H J-resolved spectrum of two isomers. Multiplet splittings with some long-range couplings are well separated in the F_1 dimension. ¹H-¹H coupling constants determined from the 2D J spectrum are listed in Table 2.

Vicinal coupling. Electron diffraction measurements²⁶ have shown that cyclohexane is a flattened chair in

Table 2.	Coupling	constants	of	cis,cis-	and	cis,trans-
	triaminocy	vclohexane*				

cis,trans	cis,cis
Vicinal: J _{af} = 3.77	$J_{\rm cd} = 3.91$
$J_{ag} = 3.77$	
$J_{\rm bf} = 4.06$	
$J_{\rm bg} = 10.72$	$J_{ci} = 11.31$
$J_{\rm bh} = 10.58$	01
Geminal: $J_{eh} = -12.32$	$J_{\rm di} = -12.76$
$J_{fg} = -13.50$	
Long-range: ⁴ J _{ei} = 1.88	

^a Concentration, 0.02 м (for each isomer) in CDCl₃; temperature, 25 °C.

which the dihedral angles are 175° (axial:axial), 55° (axial:equatorial) and 65° (equatorial:equatorial). In the perfect chair conformation (structure II), all axial protons are parallel, whereas in the flattened chair conformation (I), the carbon-proton bonds are bent outwards. Clearly, the effect of ring flattening is due to repulsive interactions between hydrogen atoms attached by axial bonds on the same side of the cyclohexane ring, so that 1,3-diaxial interactions (III) are reduced.

Garbisch and Griffith²⁷ obtained the proton spectrum of deuteriated cyclohexane with deuterium decoupling at -103 °C. With the vicinal coupling



Figure 3. Contour plot of the 2D J spectrum of the two isomeric triaminocyclohexanes. Acquisition parameters are given in the text. A 'proton-decoupled proton' spectrum is shown below the 2D J plot, and a normal high-resolution 1 H spectrum is shown above.



II

III

I

constants $(J_{aa}, J_{ae} \text{ and } J_{ee})$ and Karplus's $J-\phi$ relationship²⁴ [Equation (6)] they solved the three equations and obtained A = 12.95, n = -0.02 and $\phi = 57^{\circ}$.

$$J = A(\cos^2 \phi + n \cos \phi) \tag{6}$$

The dihedral angle (ϕ) for the vicinal (axial:equatorial) protons that they found in cyclohexane was 57°, which is consistent with electron diffraction results.²⁶

Forrest's modified Eqn (2) was used to obtain J_{calc} values which were combined with Eqns (3) and (4) to obtain dihedral angles for both isomers of triaminocyclohexane. These angles and coupling constants are listed in Table 3.

The dihedral angle ϕ_{ae} (H_d—C—C—H_c) in the *cis,cis* isomer, is calculated to be 61° (structure IV), close to the perfect chair conformation ($\phi_{ae} = 60^{\circ}$). This is due to non-bonded interaction among the three equatorial amino groups and methylene protons (which increases ϕ_{ae}). This interpretation balances the 1,3-diaxial interaction (which decreases ϕ_{ae}) in the six-membered ring and prevents the ring from being flattened. This is consistent with force field

Table 3.	Vicinal coupling constants (J_{obs} and J_{calc} , in Hz)
	and dihedral angle $\phi_{axial-equatorial}$ in cis, cis- and
	cis,trans-TACH [*]

lsomer Cis,Cis	J J _{cd}	^Ј ова 3.91	$\int_{calc}^{J_{calc}}$ for $\phi = 60^{\circ}$ 3.34	[¢] axia⊢equatorial 61
cis,trans	Jag	3.77	2.54	53
	J _{bf}	4.06	3.12	56
	Jbe	4.06	4.19	61
	J _{af}	3.77	3.42	59

^a Concentration, 0.02 M (for each isomer) in CDCl₃; temperature, 25 °C.



calculations,²⁸ which predict that *tert*-butyl equatorial substitution will force the axial proton on the same carbon toward the center of the ring, giving a dihedral angle, ϕ_{ae} , for this proton of 63°.

In the *cis,trans* isomer, the axial amino substituent causes ϕ_{ae} (H_g-C-C-H_a) to decrease to 53° (structure V) owing to strong 1,3 diaxial interaction, further flattening the six-membered ring by 4°. The other two dihedral angles are 56° for H_f-C-C-H_b and 61° for H_e-C-C-H_b, which showed that distortion induced by the equatorial amino group is much less than from the axial amino group. This is also consistent with the results of force field calculation.²⁸

Geminal and long-range coupling. Three geminal coupling constants were observed in the isomeric triaminocyclohexanes, as listed in Table 2 (-12.32, -13.50 and -12.76 Hz). The difference is evidence of a change in the H—C—H angle. It has been noted²⁷ that a decrease in the H—C—H angle makes geminal coupling more negative. As long as the triangular projection (Dreiding models) holds, the H_g—C—H_f angle is 112° (53 + 59), which is smaller than the projection angle (120°), presumably owing to the influence of the axial amino group. Thus the H_g—C—H_f geminal coupling constant has the more negative value (-13.50 Hz).

Long-range coupling, ${}^{4}J_{eq,eq}$, was also observed in *cis,trans*-triaminocyclohexane as 1.88 Hz. In general, for an unstrained saturated cyclohexane ring, it is usually in the range 1–2 Hz, and shows the maximum coupling for a perfect planar 'W' path arrangement.²⁹

Molecular dynamics

In general, when molecular motion is anisotropic (or with internal rotational modes), the Woessner



V

formulation has found much application. The T_1 value for a given carbon atom can be expressed as a function of three diffusion constants and three direction cosines:

$$1/T_1^{\rm DD} = f(R_1, R_2, R_3, l, m, n)$$
(7)

where R_1 , R_2 and R_3 are the diffusion constants describing rotational motion about three mutually perpendicular axes. The directional cosines l, m and nrepresent the angles subtended by each C-H vector with respect to the principal axes. Since three (or six) unknowns must be solved for, a molecule should be rigid and have a large number of C-H vectors asymmetrically distributed. As far as triaminocyclohexane is concerned, there are only three different C-H vectors for the cis, cis isomer and six for the cis, trans isomer (but symmetry reduces these to fewer 'independent' vectors). Assumption of isotropic overall reorientational motion for triaminocyclohexane is necessary and, from the data described below, is probably reasonable accurate.

¹³C and ¹⁵N spin-lattice relaxation data of 1,3,5-triaminocyclohexane at various pH values are given in Table 4. Diffusion constants for 1,3,5triaminocyclohexane were determined from T_1 values of the protonated carbons using MOLDYN³⁰ (Table 5). For calculations involving the simultaneous fitting of T_1 values, the largest deviations between calculated and observed values were less than 10%. ¹³C NT_1^{DD} values are determined as averages for methylene and methine groups [Eqn (8)]. The similarity of these NT_1 values is consistent with, but not proof of, essentially isotopic overall motion.

$$NT_1^{DD}(av.^{13}C) = \frac{[T_1(CH) + 2T_1(CH_2)]}{2}$$
 (8)

The approximate correlation times (τ_c) in Table 5 were calculated from Eqn (8) with average ¹³C T_1^{DD} value at different pH values. Using three p K_a values³¹ (10.4, 8.7 and 6.9), one can calculate the number of protons attached to nitrogen at different pH values; these values are used to estimate NT_1 for ¹⁵N signals.

The last column in Table 4 gives the ${}^{15}N/{}^{13}C$ dipolar NT_1 ratios. At high pH (ca 12.5), the NT_1^{DD} ratio (ca 7.5) indicates that the amino groups are all relatively rigid. The ratio increases as the pH decreases; at low pH (ca 3.5), the NT_1^{DD} ratio increases significantly, indicating rapid NH₃⁺ group rotation. Group rotation is probably facilitated by the higher inherent symmetry of the tetrahedral NH₃⁺ group, with a remaining restriction due to solvent-solute interactions.^{32,33}

Assuming rotation between three equivalent positions, the correlation time for rotation of an NH_3^+ group can be calculated in a manner analogous to that for the CH₃ group. The explicit formulation of T_1^{DD} is given by

$$\frac{1/NT_1^{DD} = (h^2 \gamma_H^2 \gamma_N^2 / r_{NH}^6) [(A/6D) + (B+C)/(6D+R_{int})] \quad (9)$$

Equation (9) assumes stochastic diffusion for the

	• ••••						-,-, 	0. ¹⁵ N	у мт D		AUT DD. 15N
Isomer	pН	C-1	C-2	C-3	C-4	NT1 DD,AV.	N-eq.	N-ax.	N-eq.	N-ax.	NT1 DD, 13C, AV.
cis,trans	12.5	2.65	1.15	2.66	1.18	2.42	7.26	9.37	14.52	18.74	7.5
	9.5	1.43	0.89	1.62	0.89	1.65	6.26	7.72	15.65	19.30	11.7
	7.5	1.42	0.80	1.45	0.74	1.49	6.15	6.20	17.22	17.34	11.6
	3.5	1.37	0.75	1.38	0.70	1.41	6.58	6.60	19.74	19.80	14.1
cis.cis	12.5	2.65	1.16		—	2.49	7.62	—	14.50		5.8
-	9.5	1.64	0.83			1.65	6.06	_	15.15		9.5
	7.5	1.42	0.80	—	_	1.50	6.15		17.22	_	11.5
	3.5	1.38	0.73	—	_	1.42	6.60		19.80	_	13.9
* <i>NT</i> ₁ valu	ies in secor	nds; conc	entratio	п, 1.0 м	(for eacl	n isomer)	in H₂O	D₂O; ter	nperature	ə, 25 °C.	

Table 5. Calculated motion properties and rotational barriers (NH_x) for triaminocyclohexane^a

		$(\times 10^{-11})$ (s)	D (×10 ⁹) (s ⁻¹)	R _{int} (× 10 ¹⁰) (s ⁻¹)		E _{act} (kcal mol ⁻¹)		
Isomer	pН			Equatorial	Axial	Equatorial	Axial	
cis.trans	12.5	1.88	8.64	1.27	2.64	4.3	3.9	
,	9.5	2.84	5.87	2.95	4.69	3.8	3.5	
	7.5	3.14	5.31	4.12	4.19	3.6	3.6	
	3.5	3.32	5.02	5. 9 4	5.98	3.3	3.3	
cis,cis	12.5	1.87	8.91	1.27		4.3		
	9.5	2.48	5.87	2.73		3.8		
	7.5	3.12	5.34	4.10		3.6		
	3.5	3.30	5.05	5.94		3.3		
^a Concentrat	tion, 1.0	м (for eac	ch isome	er) in H₂O–I	O₂O; ter	nperature 2	5℃.	

 NH_3^+ group. A, B and C are geometric constants (A + B + C = 1) and R_{int} is the internal rotation diffusion coefficient for the NH_3^+ rotation. D is the molecular diffusion constant, which is obtained from the effective correlation time $(D = 1/6\tau_c)$. The diffusion constants for internal rotation (R_{int}) of NH_x groups in Table 5 clearly show that NH_x groups rotate faster as the pH value decreases. This result is consistent with qualitative conclusions drawn from NT_1 rations between ¹⁵N and ¹³C nuclei. Using these calculated diffusion constants (R_{int}) and assuming ideal tetrahedral geometry (which may not be warranted), the energy barriers for NH_x group rotation can be calculated by the Arrhenius equation.³⁴ These results are given in the last column of Table 5.

CONCLUSION

Conformational and dynamics analysis of many compounds can be performed using the NMR techniques employed here. Traditional one- and two-dimensional NMR experiments were performed

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on a 1:1 isomeric mixture of cis, cis- and cis, trans-1,3,5-triaminocyclohexane. The proton spectrum was easily assigned using the information obtained in a 2D COSY experiment. Using Forrest's modification of the Karplus equation and the 2D SJ spectrum, the conformations of the two isomers were analysed. This analysis revealed that the ring of the cis, trans isomer is flattened due to 1,3-diaxial interactions between two amino substituents. The cis, cis isomer, on the other hand, is puckered by the non-bonded interaction between the equatorial groups and adjacent methylene protons.

The motional dynamics of the amino groups were also investigated. Using one-dimensional ¹³C and ¹⁵N spin-lattice relaxation experiments, the NH_x groups were found to rotate faster with decreasing pH. No evidence was found to indicate that the overall molecular motion was anisotropic.

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