Configuration and Conformation of the α- and β-Anomers of C-Nucleosides by Proton Magnetic Resonance Spectroscopy: New Criterion for Determination of α- and β-Anomers

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Abstract: The configuration and conformation of the α- and β-anomers of C-nucleosides, derived from formycine, were studied by ^1H NMR at 250 MHz in D_2O and in Me_2SO. It has been shown that the chemical shifts and the proton–proton vicinal coupling constants are not always reliable to distinguish the two anomers. On the other hand, proton spin–lattice relaxation, in particular that of the anomeric proton, has been found to be an excellent method for identifying the α- and β-anomers. With the exception of formycine itself, the N ⇌ S equilibrium was found to be quite dependent on the solvent, especially in the case of the α-anomers; here the N conformer is particularly predominant in D_2O. Three-spin dipolar interaction is discussed in detail to account for the relaxation of proton H(4′) of the ribose.

Numerous nucleoside analogues have been found or synthesized which have pronounced biological activities, e.g., antiviral, bactericidal, or cancerostatic. Among them, the so-called C-nucleosides, where the glycosidic linkage is a C–C bond, have encountered considerable interest. The best known of these C-nucleosides is formycine and its analogues, of which detailed crystal structures have been published.[2,3]

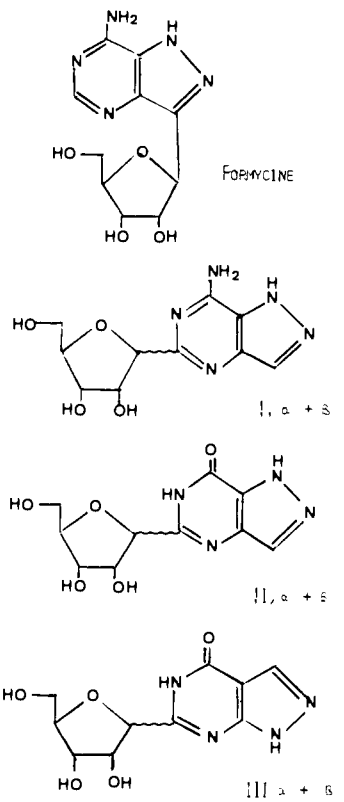
The important role of formycine prompted us to synthesize different other analogues,[4] of which the α- and β-anomers have been obtained. The determination of their anomeric configuration and of the conformation of the ribose ring poses several problems. While circular dichroism is frequently used to identify the anomers[5] because of its rapidity and economy in product, the Cotton effects of sterically unhindered nucleosides with modified chromophores may not always furnish an unambiguous assignment.[4] Crystallography is evidently the clearest, but heaviest method. Imbach's[6] NMR method requires formation of the 2′,3′-isopropylidene ether of the nucleoside, which precludes the study of the sugar conformation.

In the present communication, we have studied the configuration of formycine and certain of its analogues (see scheme) in D_2O and Me_2SO by proton magnetic resonance and spin–lattice relaxation. It is shown that α- and β-anomers can be distinguished by a rather simple relaxation test involving only the measurement of T_1 of the anomeric proton. We have chosen these analogues of formycine for three reasons (related between each other). (a) Because of the absence of a nonexchangeable proton on the base near the sugar moiety, only relaxation between close sugar protons has to be considered. The protons H(7) or H(9), respectively (see scheme), are too distant to have a measurable influence on the relaxation of the sugar protons because of the r_{ij}^{-6} relationship between relaxing protons. (b) Because of the absence of a relaxing proton on the base it can be investigated whether or not cross relaxation between sugar protons is of importance. (c) Because of the elongated C(1′)–C(2) glycosidic linkage and the absence of a substituent in the ortho position of C(2) on the base, the syn and anti conformations should be quite equally distributed and thus have little influence on the sugar conformation.

Finally, the advantages and inconveniences of NMR methods for the identification of anomers are discussed and relaxation of a three-spin system is evoked to explain some of the results.

Experimental Section

The synthesis of compounds Iα and Iβ will be published elsewhere.[28] Compounds IIα, IIβ, IIIα, and IIIβ and formycine were prepared according to ref 4.



The nucleosides were dissolved in D_2O and cleaned of eventual divalent ions by shaking with Chellex 100 and filtered. The samples were then lyophilized twice and redissolved in D_2O or Me_2SO-d_6, respectively, at a final concentration of 10^{-5} mol/mL. These solutions were introduced into NMR tubes, degassed in a vacuum line, and sealed.

The ^1H NMR spectra were recorded on a CAMECA TSN-250 spectrometer (250 MHz). The apparatus operated in the pulsed-Fourier transform mode associated to a 24K Nicolet computer (8K program, 16K data). The proton relaxation measurements were carried out in Fourier transform by the inverse recovery method. For the

Table I. Computed Values of the Sum $\Sigma r_{ij}^{-6} \times 10^2$ (Å^{-6}) for Two Conformers N ($P_N = 15°$) and S ($P_S = 165°$) of the α- and β-Anomers

	H(1′)	H(2′)	H(3′)	H(4′)	H(5′)	H(5″)
N(α)	0.999	1.390	1.408	1.454	4.675	4.653
S(α)	0.735	1.505	1.527	1.570	4.909	4.780
N(β)	0.434	0.990	1.346	1.383	4.719	4.693
S(β)	0.314	1.150	1.639	1.515	5.00	4.854

coupling measurements, an acquisition time of 5 s was used (resolution $\simeq$ 0.2 Hz).

NMR Methods to Study α- and β-Anomers

(a) Chemical Shifts and Vicinal Coupling Constants. These two parameters are the most widely used ones. Frequently, they are compared with a closely related nucleoside, the anomeric configuration of which is known. There are, however, many factors which can influence the electronic environment of a proton, and thus its chemical shift. Similarly, the coupling constants are quite sensitive to conformational changes of the ribose. Thus these two parameters are not always reliable indications if only one anomer is available.

Imbach[6] has observed that the difference of chemical shifts ($\Delta\delta$) of the methyl groups of 2′,3′-isopropylidene nucleosides was different for the two anomers: $\Delta\delta = 0.21$ ppm for the β-anomer and $\Delta\delta = 0.10$ ppm for the α-anomer. Apart from the fact that the formation of the 2′,3′-isopropylidene bridge precludes conformational analysis of the sugar, it implies a supplementary reaction.

The fact that in β-nucleosides, $J_{1'2'} + J_{3'4'} = 10 \pm 1$ Hz permits in some cases to distinguish between the α- and β-anomers,[7,8] since in α-nucleosides the sum $J_{1'2'} + J_{3'4'}$ can vary from 10 to 13 Hz. In the pseudorotational treatment of Altona and Sundaralingam[9] for each vicinal coupling constant an equation $J_{ij}(\text{obsd}) = X_n J_{ij}^N + X_s J_{ij}^S$ describing the N $\rightleftharpoons$ S equilibrium is established, where $X_s = 1 - X_n$ and J_{ij}^N and J_{ij}^S are the coupling constants in the pure N and S form. A solution for X_n is sought which will satisfy all observed coupling constants, giving a unique set of pseudorotation parameters P^N, P^S, and τ_m. For the β-ribosides, simple graphical methods had been described.[10,11] Since the protons of the α-ribosides (and other nucleosides except β-ribosides) are not symmetrically arranged, the problem is more complex. Still from the two cis vicinal coupling constants $J_{1'2'}$ and $J_{2'3'}$ the pseudorotational parameters P^N, P^S, and τ_m can be obtained, and from the trans coupling $J_{3'4'}$ the fractions X_n and X_s are computed (see Table V1). A general analysis of furanose conformations, including α-ribosides, will be published elsewhere.

(b) Nuclear Overhauser Effect (NOE). In a molecule containing several protons the magnetic relaxation of a given proton in solution is due to dipolar interaction with other protons and mainly with the nearest one. The dipolar interaction decreases with the sixth power of the interproton distance. The saturation of a neighboring proton leads to an increase of the polarization of the proton considered and thus to an increase of the signal:[12,13]

$$\xi_{ij}/\xi_{ik} = (r_{ij}/r_{ik})^{-6} \tag{1a}$$

$$\sum_{j \neq i} \xi_{ij} = 0.5 \tag{1b}$$

where ξ_{ij} is the NOE of proton i when proton j is saturated.

In the case of N-nucleosides, for the nonexchangeable protons of the bases (H(8) of purines, H(6) of pyrimidines) one can obtain considerable NOE when saturating H(2′) and H(3′) in the β-anomers,[14-18] but small or negligible NOE for the α-anomers. In the case of the C-nucleosides studied, the nonexchangeable proton of the base is far from the ribose ring, one could only observe the NOE of H(1′) when H(2′) is saturated. The coupling between these two protons further complicates NOE measurements.

(c) Relaxation. Recent proton and ^{13}C relaxation measurements demonstrated[19-24] that the reorientation of the whole molecule is very much faster than intramolecular movements and that the relaxation of any proton depends on only one correlation time τ_c. Furthermore, cross relaxation can be assumed to be negligible; i.e., dipolar interaction between two spins I and J is not perturbed by the presence of other protons in the neighborhood. This means that in the computation of the relaxation time of a given proton, the contributions of all other atoms are summed (two spin system). The return to equilibrium of longitudinal magnetization is thus an exponential function of t.

$$I_z = I_0(1 - 2e^{-t/T_1}) \tag{2}$$

$$T_1^{-1} = \tfrac{3}{10}\gamma^4\hbar^2 \sum r_{ij}^{-6} \cdot f(\tau_c) \tag{3a}$$

where

$$f(\tau_c) = \frac{\tau_c}{1 + \omega^2\tau_c^2} + \frac{4\tau_c}{1 + 4\omega^2\tau_c^2} \tag{3b}$$

For small molecules in solution at room temperature $\omega^2\tau_c^2 \ll 1$ and thus $f(\tau_c) = 5\tau_c$

$$T_1^{-1} = \tfrac{3}{2}\gamma^4\hbar^2\tau_c \sum r_{ij}^{-6} \tag{4}$$

In the absence of any knowledge of the anomeric configuration of C(1′) and thus of the pseudorotational parameters, these enter as unknowns in our computations. The strategy consists in the computation of Σr_{ij}^{-6} for the two conformers N and S of the α- and β-anomers. From the experimental relaxation time T_1 the correlation time τ_c is calculated for the N and S conformer of each proton. The optimal value of the N/S ratio is found where the mean error $\Delta\tau_c/\bar{\tau}_c$ is a minimum.

In Table I are shown the computed values of the sum Σr_{ij}^{-6} using typical conformational parameters ($P_N = 15$, $P_S = 165$, $\tau_m = 40°$). We have found that the values of Σr_{ij}^{-6} are rather insensitive to reasonable variations of the pseudorotational parameters ($\Delta P = \pm 20°$, $\Delta\tau_m = \pm 10°$). The computations have been performed with the coordinates of α-pseudouridine[25] and formycine.[2] It should be noted that the sum Σr_{ij}^{-6} for H(1′) of α-nucleosides are more than twice as large as those of the β analogues. The T_1 (H(1′)) values of β-nucleosides should thus be more than twice as large than those of α-nucleosides. A simple measurement of the relaxation times of the anomeric proton (H(1′)) should thus unambiguously distinguish between the α- and β-anomers, without having recourse to a comparison with the other analogue. A similar criterion to distinguish α- and β-anomers of pyranoses has been described by Preston and Hall.[26]

Results and Discussion

(1) Chemical Shifts. Chemical shifts of the nucleosides studied have been measured in D_2O (DSS as internal reference) and in Me_2SO (Me_4Si as internal reference) and are summarized in Tables II and III. In a given solvent, the chemical shifts of a given anomer are quite similar for the whole family. Figures 1 and 2 show the 250-MHz spectra of Iα and Iβ in the two solvents.

Table II. Proton Chemical Shifts (in parts per million from DSS) in D_2O at 22 °C[a]

Nucleoside	H(2)(H(8))	H(1′)	H(2′)	H(3′)	H(4′)	H(5′)	H(5″)
Formycine	8.186	5.221	4.607	4.357	4.231	3.912	3.801
Iα[26]	8.119	5.165	4.613	4.374	4.214	3.952	3.771
Iβ	8.119	4.839	4.344	4.293	4.188	3.989	3.824
$\Delta\delta(H_\alpha - H_\beta)$		0.326	0.269	0.081	0.026	−0.037	−0.053
IIα	8.092	5.150	4.581	4.383	4.234	3.967	3.764
IIβ	8.120	4.862	4.385	4.269	4.196	4.004	3.857
$\Delta\delta(H_\alpha - H_\beta)$		0.288	0.196	0.114	0.038	−0.037	−0.093
IIIα	8.269	5.186	4.614	4.384	4.232	3.967	3.760
IIIβ	8.287	4.915	4.410	4.263	4.197	4.002	3.849
$\Delta\delta(H_\alpha - H_\beta)$		0.271	0.204	0.121	0.035	−0.035	−0.089

[a] They were recorded to better than 0.005 ppm.

Table III. Proton Chemical Shifts of α- and β-Anomers (in parts per million relative to Me_4Si) in Me_2SO at 42 °C[a]

Nucleoside	H(2)(H(8))	H(1′)	H(2′)	H(3′)	H(4′)	H(5′)	H(5″)
Formycine	8.145	4.950	4.497	4.101	3.937	3.647	3.502
Iα	8.073	4.875	4.317	3.965	3.996	3.535	3.447
Iβ	8.035	4.671	4.140	4.087	3.909	3.726	3.522
$\Delta\delta(H_\alpha - H_\beta)$		0.204	0.177	−0.122	0.087	−0.191	−0.075
IIα	8.007	4.859	4.346	4.096[b]	4.081[b]	3.598	3.465
IIβ	8.070	4.615	4.144	4.031	3.944	3.742	3.601
$\Delta\delta(H_\alpha - H_\beta)$		0.244	0.202	0.065	0.137	−0.144	−0.136
IIIα	8.041	4.880	4.354	4.094[b]	4.082[b]	3.621	3.471
IIIβ	8.111	4.626	4.140	4.023	3.940	3.747	3.598
$\Delta\delta(H_\alpha - H_\beta)$		0.254	0.214	0.071	0.142	−0.126	−0.127

[a] They were generally recorded to better than 0.005 ppm. [b] Determined to 0.010 ppm.

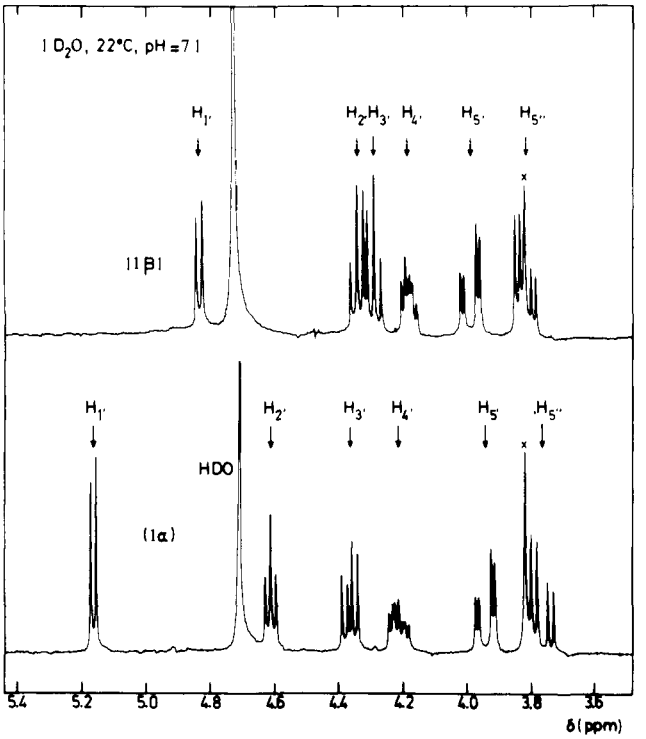


Figure 1. ^1H NMR spectrum (250-MHz) of Iα and Iβ in D_2O (22 °C, pH 7). Only the portion of the spectra involving the nonexchangeable protons of the ribose is shown.

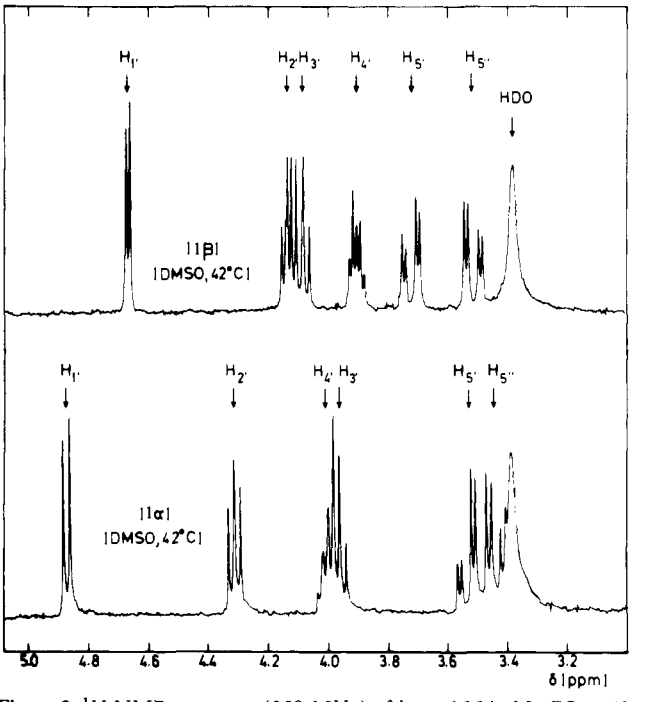


Figure 2. ^1H NMR spectrum (250-MHz) of Iα and Iβ in Me_2SO at 42 °C.

The chemical shifts of the α-anomers are frequently between those of formycine (a β-anomer) and those of the corresponding β-nucleoside; in many cases the chemical shifts of the α-anomer are very close to those of formycine.

The difference $\Delta\delta$ ($H_i^\alpha - H_i^\beta$) is quite important and decreases the farther away the proton is from the anomeric carbon (C(1′)). While the ribose protons of the α-anomers are at lower field than those of the β-anomer, the inverse is true for H(5′) and H(5″). Thus the whole ribose proton spectrum of the β-nucleosides is packed within 1.1 ppm; it is spread over 1.5 ppm in the α-anomers.

A priori, no decision can be made on the anomeric configuration on the basis of chemical shifts alone.

(2) Coupling Constants. Tables IV and V summarize the coupling constants in D_2O and Me_2SO, respectively. Only

Table IV. Vicinal Coupling Constants (in hertz) and Rotamer Distributions of α- and β-Anomers in D_2O at 22 °C[a]

Nucleoside	$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$J_{4'5'}$	$J_{4'5''}$	$J_{5'5''}$	P_{gg}	P_{gt}	P_{tg}
Formycine	7.6	5.3	3.4	3.1	3.8	12.8	0.64	0.22	0.14
Iα	3.8	4.5	8.2	2.7	4.6	12.5	0.59	0.32	0.09
Iβ	4.6	5.3	5.4	2.8	3.8	12.5	0.68	0.22	0.10
IIα	3.8	4.2	8.7	2.7	4.9	12.5	0.55	0.36	0.09
IIβ	4.2	4.9	6.0	3.0	3.4	12.5	0.71	0.17	0.12
IIIα	3.8	4.2	8.7	2.7	4.7	12.5	0.58	0.33	0.09
IIIβ	3.8	4.9	6.5	2.7	3.0	12.5	0.79	0.12	0.09

[a] They were determined with an accuracy of 0.2 Hz.

Table V. Vicinal Coupling Constants (in hertz) and Rotamer Distributions of α- and β-Anomers in Me_2SO at 42 °C[a]

Nucleoside	$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$J_{4'5'}$	$J_{4'5''}$	$J_{5'5''}$	P_{gg}	P_{gt}	P_{tg}
Formycine	7.2	5.3	2.9	3.0	3.4	12.1	0.71	0.17	0.12
Iα	5.3	4.9	5.3	3.6	4.6	11.8	0.48	0.32	0.20
Iβ	3.4	4.9	6.0	3.0	3.4	11.8	0.71	0.17	0.12
IIα	5.2	3.6	5.8[b]	3.0	3.8	12.0	0.66	0.22	0.12
IIβ	4.2	4.9	5.7	3.0	2.4	11.8	0.83	0.05	0.12
IIIα	4.6	3.9	5.8[b]	2.5	3.8	11.8	0.72	0.22	0.06
IIIβ	4.2	4.9	5.7	3.0	2.3	11.8	0.84	0.04	0.12

[a] They were generally determined with an accuracy of 0.2 Hz. [b] Determined to 0.3 Hz.

Table VI. Conformational Parameters of Nucleosides Studied in D_2O and Me_2SO[a]

Compd	Solvent	P^N	P^S	τ_m	X_S	X_N
Formycine	D_2O	24	156	43.5	0.70	0.30
	Me_2SO	12	168	41	0.715	0.285
Iα	D_2O	−23	167	49.5	0.05	0.95
	Me_2SO	−13	167	43	0.34	0.66
Iβ	D_2O	10	170	40.5	0.46	0.54
	Me_2SO	−3	183	42	0.36	0.64
IIα	D_2O	−20	160	48	0.05	0.95
	Me_2SO	0	180	50	0.45	0.55
IIβ	D_2O	9	171	42.5	0.41	0.59
	Me_2SO	6	174	42.5	0.425	0.575
IIIα	D_2O	−20	160	48	0.05	0.95
	Me_2SO	−10	170	49	0.41	0.59
IIIβ	D_2O	12	168	43.5	0.37	0.63
	Me_2SO	6	174	42.5	0.425	0.575

[a] For the pseudorotation treatment the Karplus equation with $A = 10.2$, $B = -1.2$, $C = 0$ was used.

formycine shows a high $J_{1'2'}$ value (7.6 Hz in D_2O and 7.2 Hz in Me_2SO), while they are considerably lower in all the other nucleosides. It is noteworthy that an important solvent effect is observed not only on the chemical shifts, but also on the coupling constants, indicating a shift in the conformational equilibrium: in D_2O, $J_{1'2'}$ is lower in the α-anomer, while the inverse is true in Me_2SO; $J_{3'4'} \approx 6.0$ Hz is the same for the α and β analogues in Me_2SO, but in D_2O this value is >8 Hz for the α analogues and lower for the β-anomers.

(3) Conformation of Ribose Ring and Exocyclic Group. The strategy to extract conformational parameters of α-ribosides from proton–proton coupling constants has been evoked above. The pseudorotational parameters thus obtained are summarized in Table VI. While the S conformation is largely preponderant in formycine independent of the solvent used, this is not the case for the other compounds studied. Note that the crystal structures of formycine,[2b] its bromohydrate,[2a] and its oxo analogues[3] are all in the S conformation.

In contrast to formycine, the other compounds studied show a very large N contribution in D_2O with some unusual phase angle P^N in the α-nucleosides. In Me_2SO the N conformation is still predominant, but to a much lesser extent, while the phase and puckering angles (τ_m) remain essentially constant when going from D_2O to Me_2SO.

The strong solvent effect on the ribose conformation is in contrast with the rather small variation of the exocyclic rotamer distribution (Tables IV and V).

(4) Spin–Lattice Relaxation. Due to the different solubility of the α- and β-anomers in D_2O and Me_2SO, the interference between ribose protons and the presence of the HDO peak, the relaxation times of the α- and β-anomers were measured at 22 °C in D_2O and at 42 °C in Me_2SO. By this choice of temperature (temperature effect on the viscosity), correlation times τ_c in Me_2SO are comparable with those in D_2O, which facilitates comparison of T_1 values between the two anomers. In principle, the determination of the anomeric configuration by relaxation is independent of the solvent and temperature chosen.

The relaxation of the nonexchangeable base proton is rather long (between 12 and 17 s). Since the proton is far from the sugar protons, its relaxation is probably also influenced by other causes than the dipolar relaxation of intramolecular protons. It was therefore not studied in detail.

The spin–lattice relaxation times T_1 of the ribose protons

Table VII. Experimental and Calculated Relaxation Times (in seconds, first and second line, respectively) of α- and β-Anomers

Nucleoside	Solvent	H(1′)	H(2′)	H(3′)	H(4′)	H(5′)	H(5″)	$\bar{\tau}_o \times 10^{10}$ s
Formycine	Me_2SO	4.04	1.32	1.10	1.32	0.34	0.34	1.02
	42 °C	4.18	1.47	1.05	1.22	0.30	0.31	
Iα	D_2O	1.60	1.07	1.31	1.33	0.45	0.45	0.95
	22 °C	1.56	1.06	1.19	1.36	0.34	0.35	
Iβ	Me_2SO	3.33	1.37	1.37	1.38	0.40	0.40	1.01
	42 °C	3.49	1.53	1.23	1.33	0.32	0.32	
IIα	D_2O	1.54	1.08	1.06	1.60	0.40	0.40	0.96
	22 °C	1.55	1.05	1.16	1.33	0.29	0.30	
IIβ	Me_2SO	3.35	1.38	1.23	1.29	0.35	0.35	1.04
	42 °C	3.38	1.48	1.22	1.29	0.31	0.31	
IIIα	D_2O	1.35	1.05	1.10	1.20	0.45	0.45	1.06
	20 °C	1.43	0.95	1.06	1.21	0.29	0.31	
IIIβ	Me_2SO	3.24	1.39	1.21	1.24	0.36	0.36	1.08
	42 °C	3.27	1.43	1.18	1.25	0.30	0.30	

of the seven nucleosides studied are presented in Table VII. As expected, the relaxation time of the H(1′) proton of the β-anomers is about two and a half times longer than that of the α-anomer, which in turn is not very different from the T_1's of the other protons of the ribose ring. *This criterion by itself permits the unambiguous determination of the anomeric configuration, even if only one anomer is available.* In the general case, if the base contains a nonexchangeable proton close to the ribose ring (e.g., H(8) in the natural purine nucleosides, or H(6) in the pyrimidine nucleosides), the relaxation time T_1 of the ribose protons will also depend on the anti-syn equilibrium around the base as had been demonstrated previously by NOE[14-18] and relaxation measurements.[19-24] The presence of this base proton reduces slightly T_1 of proton H(1′), H(2′), and H(3′), particularly in the β-anomers; consequently, the ratio T_1 (H(1′))/T_1 (H$_i$) (i = 2′, 3′, 4′) of the β-anomers will change only little and is sensibly the same, between 2.4 and 3.0,[19] while the same ratio of the α-anomers is always below 1.5.

The distinction between α- and β-anomers can be simplified in the following way. Equation 2 describes the return to equilibrium of longitudinal magnetization when the method of inversion recovery is used: $I_z = 0$, if $\tau_o = T_1 \ln 2$. For the β-nucleosides studied T_1 (H(1′)) > 3.2 s and therefore $\tau_o > 2.2$ s, while for the α-anomers T_1 (H(1′)) < 1.6 s, and $\tau_o < 1.1$ s. *Thus if $\tau \simeq 1.5$ s, the H(1′) signal will be positive for an α-anomer, and negative for a β-anomer.* Thus, instead of measuring the relaxation time T_1, which takes several hours, this test can be used which takes only a few minutes. In the case of the natural nucleosides, where a base proton is close to the ribose ring, T_1 (H(1′)β) > 2 s at room temperature,[19] τ of about 1.0–1.2 s is appropriate to distinguish the α- and β-anomers.

Apart from the determination of anomeric configuration, spin-lattice relaxation measurements in principle permit conformational analysis, in our case the determination of the N/S ratio. This also permits one to verify whether computations based on the assumption of two spin relaxation are valid for all protons of the ribose ring. For this purpose, the correlation time τ_c is calculated for each proton as a function of the fraction X_n and X_s, using the relaxation times measured. Figure 3a shows the typical case of formycine.

We have found that for all nucleosides studied, α- and β-anomers, one can always find a value of X_n or X_s ($= 1 - X_n$) for which the τ_c's agree to better than 10% between each other. The only proton which shows exceptions is H(4′), where τ_c is always 20–40% lower than the mean $\bar{\tau}_c$ of the other protons. We have also observed that this difference is increasing with an increase in the gauche-gauche population around the C(4′)-C(5′) bond. This indicates that the relaxation of proton H(4′) does not follow the two-spin case, particularly in the gauche-gauche conformation of the exocyclic group; the two other conformers follow the two-spin treatment within the experimental error.

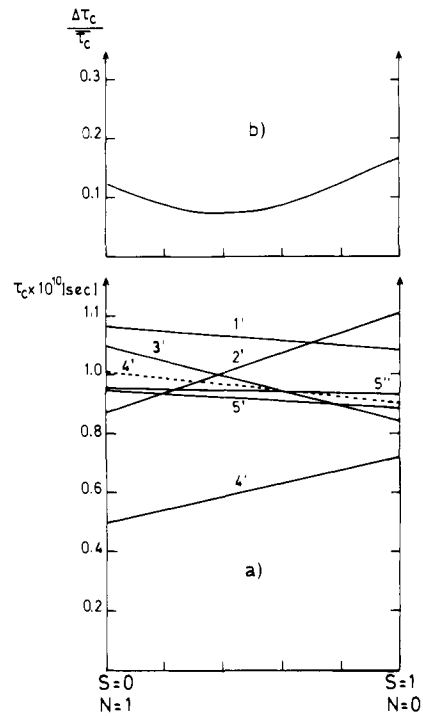


Figure 3. (a) Correlation time τ_c of the ribose protons as a function of N and S proportions in the typical case of formycine. The broken line has been obtained for H(4′) by taking into account the relaxation of a three-spin system in the gauche-gauche rotamer. (b) $\Delta\tau_c/\bar{\tau}_c$ computed for the four protons 1′, 2′, 3′, and 4′ of formycine, as a function of N and S proportions.

In the Appendix is elaborated the treatment of a three spin system,[23,27] which in our case is due to the dipolar relaxation of H(4′) by H(5′) and H(5″). When using the new value of Σr_{ij}^{-6} for the computation of τ_c (H(4′)), very good agreement is obtained with the correlation times of the other protons (broken line in Figure 3a). Figure 3b gives the ratio of $\Delta\tau_c/\bar{\tau}_c$ computed for the four protons 1′, 2′, 3′, and 4′ of the ribose ring as a function of the N and S proportions for formycine. A very shallow minimum around 40% S conformer is obtained, while from the coupling constants (Table VI), ~70% S conformer is computed.

The discrepancy between the two methods is due to several causes. The ratio $\Delta\tau/\bar{\tau}_c$ is not very sensitive to changes in the conformation of the ribose ring, since the difference in Σr_{ij}^{-6} for N and S conformers are small (≃25%, Table I). On the other hand the experimental error in T_1 measurements is about

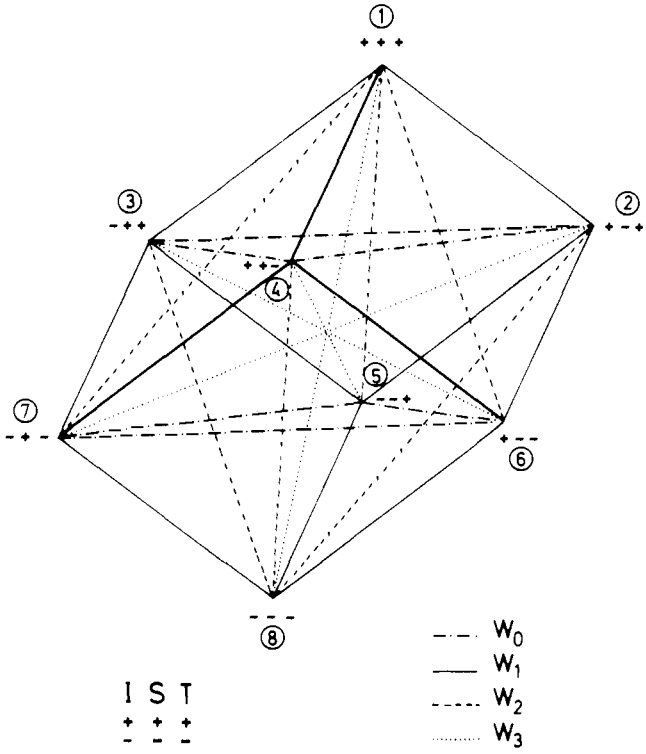


Figure 4. Transition possibilities for I, S, and T three-spin one-half system.

10% (±5%). The combination of these two effects can therefore provoke larger variations in the ratio of N and S conformers.

Conclusion

While the relaxation of a ^{13}C atom depends only on the number of directly attached protons, the relaxation of a proton in turn depends on the neighboring protons and thus gives information on structure and conformation of the molecule. An experimental error of 10% in the T_1 measurement has only small incidence on the interatomic distance. The fact that proton spin–lattice measurements can be performed in relatively dilute concentrations reduces intermolecular interactions. In the case of nucleosides, proton–proton spin–lattice relaxation had been shown to be an excellent method to study the conformation around the glycosidic linkage,[19-24] and in this paper to distinguish without ambiguity the anomeric configuration of the C(1′) atom. It is, however, relatively insensitive to conformational changes of the ribose ring and does not permit the determination of the N/S ratio.

Appendix. Treatment of Relaxation Involving Three Spins

The dipolar interaction of a two-spin system has been treated in detail by Solomon.[12] An analogous method is here applied to the case of a three-spin system. In Figure 4 the different transition possibilities of a system of three spins, I, S, and T, are illustrated.

The time-dependent evolution of the proportion N_i of level i (= 1, 2, 3, . . . , 8) and of the longitudinal magnetization of each spin is expressed by the following equations:

$$\frac{dN_i}{dt} = \sum_{j}^{j\neq i} (-N_i + N_j)W_{ij} + \text{Cte} \qquad \text{(A1)}$$

$$dI_z/dt = -I_z(2W_{13} + W_{15} + W_{17} + W_{23} + W_{34}) - S_z(W_{15} - W_{23}) - T_z(W_{17} - W_{34}) + \text{Cte} \qquad \text{(A2a)}$$

$$dS_z/dt = -I_z(W_{15} - W_{23}) - S_z(2W_{12} + W_{23} + W_{38} + W_{15} + W_{24}) - T_z(W_{38} - W_{24}) + \text{Cte} \qquad \text{(A2b)}$$

$$dT_z/dt = -I_z(W_{17} - W_{34}) - S_z(W_{38} - W_{24}) - T_z(2W_{14} + W_{24} + W_{17} + W_{38} + W_{34}) + \text{Cte} \qquad \text{(A2c)}$$

Where W_{ij} is the transition probability per unit time between state i and state j,

$$W_{ij} = \lim_{t\to\infty} \frac{1}{\hbar^2 t} \left| \int_0^t \langle \mathcal{H}_{ij}'(t') \rangle\, e^{-i\omega_{ij}t'} dt' \right|^2 \qquad \text{(A3)}$$

where $\langle\ \rangle$ signifies the mean statistical value and $\mathcal{H}'$ is the Hamiltonian of dipolar interaction.

$$\mathcal{H}' = \gamma^2\hbar^2 \sum_{i,j}^{i\neq j} \frac{\mathbf{I}_i\mathbf{I}_j}{r_{ij}^3} - \frac{3(\mathbf{r}_{ij}\cdot\mathbf{I}_i)(\mathbf{r}_{ij}\cdot\mathbf{I}_j)}{r_{ij}^5} \qquad \text{(A4)}$$

The transition probabilities W_{ij} can be classed into four groups;

(a) W_1 (e.g., W_{12}, W_{25}, . . .), a single spin changes sign: $|-\rangle \rightarrow |+\rangle$, or inversely.

$$W_1^I = \frac{3}{20}\gamma^4\hbar^2 \left(\frac{1}{r_{IS}^6} + \frac{1}{r_{IT}^6}\right) \frac{\tau_c}{1+\omega^2\tau_c^2} \qquad \text{(A5a)}$$

(b) W_2 (e.g., W_{16}, W_{17}, . . .), one spin remains unchanged, while the two other spins of equal sign change *simultaneously* from state $|--\rangle$ to state $|++\rangle$, or inversely.

$$W_2^{IS} = \frac{3}{5}\gamma^4\hbar^2 \left(\frac{1}{r_{IS}^6}\right) \frac{\tau_c}{1+4\omega^2\tau_c^2} \qquad \text{(A5b)}$$

(c) W_0 (e.g., W_{23}, W_{34}, . . .), one spin remains unchanged, while the two other spins of opposite sign change *simultaneously* their sign: $|+-\rangle \rightarrow |-+\rangle$, or inversely.

$$W_0^{IS} = \frac{\gamma^4\hbar^2}{10} \left(\frac{1}{r_{IS}^6}\right) \tau_c \qquad \text{(A5c)}$$

(d) The probability W_3 in which three spins change their sign simultaneously is negligible or nil.

Case of Two Identical Spins. One supposes that spin I is equidistant from S and T, i.e., $r_{IS} = r_{IT}$ (case of interaction of H(4′) with H(5′) and H(5″) in the gauche–gauche rotamer). Under the conditions where $\omega^2\tau_c^2 \ll 1$, eq A2 becomes:

$$\frac{dI_z}{dt} = -2aI_z - \frac{a}{2}(S_z + T_z) + \text{Cte} \qquad \text{(A6a)}$$

$$\frac{d(S_z + T_z)}{dt} = -aI_z - (\tfrac{3}{2}b + a)(S_z + T_z) + \text{Cte} \qquad \text{(A6b)}$$

Where $a = (\gamma^4\hbar^2/r_{IS}^6)\tau_c$ and $b = (\gamma^4\hbar^2/r_{ST}^6)\tau_c$

$$I_z = Ae^{-\lambda_1 t} + Be^{-\lambda_2 t} \qquad \text{(A7)}$$

where:

$$\lambda_2^1 = -\frac{3}{2}\left(a + \frac{b}{2}\right) \pm \frac{1}{2}\sqrt{3\left[\left(a - \frac{b}{2}\right)^2 + \frac{b^2}{2}\right]} \qquad \text{(A8)}$$

In the case of the gauche–gauche rotamer, the term λ_2 is about 10 times larger than λ_1 and therefore $e^{-\lambda_2 t}$ is negligible compared with $e^{-\lambda_1 t}$. If we take into account the initial conditions, we obtain:

$$I_z = I_0(1 - 2e^{-\lambda_1 t}) \qquad \text{(A9)}$$

where

$$\lambda_1 = \frac{1}{T_1(\text{H}(4'))} = \gamma^4\hbar^2\tau_c \left\{\frac{3}{2}\left(\frac{1}{r_{4'5'}^6} + \frac{1}{2r_{5'5''}^6}\right) - \frac{1}{2}\sqrt{3\left[\left(\frac{1}{r_{4'5'}^6} - \frac{1}{2r_{5'5''}^6}\right)^2 + \frac{1}{2}\left(\frac{1}{r_{5'5''}^6}\right)^2\right]}\right\} \qquad \text{(A10)}$$

Among the three exocyclic rotamers, only the gauche–gauche contribution has to be corrected in this way.

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Ring Conformation and Barrier to Inversion of 1,3-Disilacyclobutane from Low-Frequency Vibrational Spectra[1]

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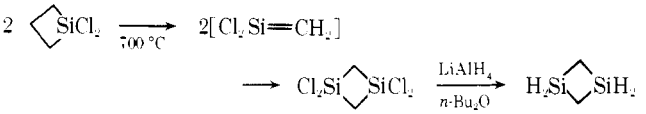
Abstract: The far-infrared and low-frequency Raman spectra of 1,3-disilacyclobutane and the far-infrared spectrum of 1,3-disilacyclobutane-*1,1,3,3-d*$_4$ have been recorded and analyzed. The spectra consist of a large number of bands between 30 and 200 cm^{-1} which correspond to transitions involving the excited states of the ring-inversion vibration. The potential energy function for this vibration was determined from the transition frequencies, and it establishes the equilibrium conformation of the ring. In the ground state, the molecule is puckered with an average dihedral angle of 24°. The barrier to inversion is 87 cm^{-1} (0.25 kcal/mol). A study of the isotopic shift in going from the d_0 to the d_4 molecule demonstrates that the SiH_2 in-phase rocking motion is coupled to the ring-inversion vibration. The syntheses of 1,3-disilacyclobutane, 1,3-disilabutane, and some related compounds are also described.

Low-frequency vibrational spectroscopy has been effectively used during the past ten years for determining the conformations and energy barriers to inversion of small ring systems.[2-7] One of the systems examined was silacyclobutane (and its 1,1-d_2 derivative), which Laane and Lord[8] showed from far-infrared studies to have a puckered ring with a dihedral angle of 35° and a barrier to inversion of 440 cm^{-1} (1.26 kcal/mol). Vapor-phase Raman spectra[7] confirmed these results. Since similar data had also been reported for cyclobutane[9-11] (dihedral angle: 33°; barrier to inversion: 515 cm^{-1}), 1,3-disilacyclobutane (and its 1,1,3,3-d_4 derivative) was prepared so that data for the three different $MH_2CH_2M'H_2CH_2$ systems (where M and M' are C or Si) could be used to better evaluate the intramolecular forces responsible for the conformations of these molecules.

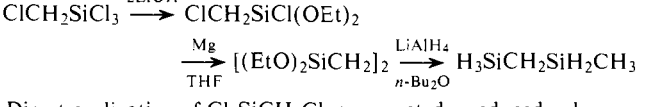
In this report, we present the far-infrared and Raman ring-puckering spectra and the vibrational potential energy function governing the ring-inversion process for 1,3-disilacyclobutane. Calculations have been carried out on the kinetic energy (reduced mass) expansions for both molecules in order to reproduce the observed frequency shifts upon isotopic substitution and to obtain a valid representation of the ring-inversion motion. The evaluation of the degree of mixing of the SiH_2 (or SiD_2) rocking motion into the inversion coordinate was of special interest. The results obtained complement our previous studies on other four- and five-membered ring silicon systems.[7,8,12-18]

Experimental Section

Synthetic Methods. 1,3-Disilacyclobutane was prepared by the $LiAlH_4$ reduction of 1,1,3,3-tetrachloro-1,3-disilacyclobutane, which was obtained by pyrolyzing 1,1-dichloro-1-silacyclobutane[19] at 700 °C according to methods described by Nametkin and co-workers.[20] This type of pyrolysis reaction is of considerable interest in that it is postulated to go through the $[Cl_2Si{=}CH_2]$ intermediate.[21]



An attempt to prepare 1,3-disilacyclobutane by reducing 1,1,3,3-tetraethoxy-1,3-disilacyclobutane with $LiAlH_4$ resulted instead in the ring-cleaved product, 1,3-disilabutane:



Direct cyclization of Cl_3SiCH_2Cl, as expected, produced only very poor yields (~2%) of 1,1,3,3-tetrachloro-1,3-disilacyclobutane.

1,1,3,3-Tetrachloro-1,3-disilacyclobutane. 1,1-Dichloro-1-silacyclobutane, prepared as previously described,[19] was pyrolyzed according to the procedure of Nametkin and co-workers[20] using a Vycor tube packed with porcelain chips and heated to 700 °C. The starting material was introduced at the rate of 3 mL/h and the pyrolysis products were collected in a series of cold temperature traps. Distil-