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Proton Nuclear Magnetic Relaxation: An Application to the Study of the Conformation and Configuration of Saframycin A

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Proton nuclear magnetic resonance (^{1}H-NMR) relaxation of saframycin A in C_6D_6 has been studied. Quantitative analysis of spin-lattice relaxation times and nuclear Overhauser effect (NOE) factors allowed the determination of the α-axial orientation of the nitrile group at C_{21}, (previously unknown). The conformation of saframycin A in C_6D_6, which was deduced from the relaxation data, was compared with that of saframycin C in the crystal state.

Keywords——saframycin A; stereochemistry; ^{1}H-NMR; spin-lattice relaxation time; NOE difference spectrum; distance geometry approach

Introduction

Saframycin A is a member of the saframycins isolated from the streptothricin-producing strain of *Streptomyces lavendulae*.[1-3] Saframycins exhibit wide-spectrum antimicrobial activity, and saframycin A is particularly important because it exhibits strong antitumor activities by direct interaction with deoxyribonucleic acid (DNA).[2-5] The structure of saframycin A has already been elucidated by carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy and chemical reaction[6] but the orientation of the nitrile group at C_{21} remained unknown. To determine this configuration and the conformation of the whole molecule in liquids, Lown *et al.* carried out extensive analysis of the 400 MHz ^{1}H-NMR spectra of saframycin A by conventional techniques,[7] but their analysis could not establish the configuration at C_{21} and was unable to provide information about the molecular conformation in liquids. Establishing the conformation as well as the configuration of saframycin A is an interesting problem in relation to an understanding of the molecular recognition process between saframycin A and DNA.[4,7] Meanwhile, X-ray analysis of saframycin A has been unsuccessful to date.

These days proton spin-lattice relaxation times (T_1) can be obtained with relative ease because of improvements in instruments. Several authors[8] have successfully applied this

quantity to conformational and configurational problems by the use of a quantitative T_1 treatment, its usefulness in such problems having been suggested by Hall.[9] This stimulated us to apply this method to the determination of the orientation of the nitrile group of saframycin A as well as the conformation of the flexible pyruvamide side chain. As suggested by Colebrook *et al.*,[10] the normalized relaxation rate may be useful for discrimination of epimeric pairs in a qualitative manner, when both of the epimeric pair are available. In the present case, unfortunately, only one of the epimeric pair was available and so their method was not applicable. In this paper relaxation data are treated quantitatively on the basis of generally accepted assumptions; overall isotropic tumbling of the molecule and an intramolecular dipole–dipole interaction mechanism. The calculated T_1 values for the two epimers with α-axial or β-equatorial orientation of the nitrile group at C_{21} were compared with observed values, which may allow discrimination of the correct configuration. In addition to the analysis of T_1 data, the nuclear Overhauser effect (NOE) was observed by means of the difference spectral technique,[11] which is rapidly becoming an important tool for organic structural elucidation and conformational analysis because of its high sensitivity and selectivity compared with the conventional method. Quantitative treatments of NOE factors were also made to confirm the interpretation of the observed NOE.

For quantitative treatment of relaxation data, we developed a program named of RSCA, capable of executing calculations of molecular coordinates and of the corresponding relaxation parameters.

We were thus able to establish the configuration of the nitrile group at C_{21} as α-axial, and the conformation of saframycin A in C_6D_6 was reinvestigated.

Experimental

Materials——Saframycin A was dissolved in C_6D_6 and the solution was degassed by freeze–pump–thaw cycles. To replace the NH proton by deuterium, lyophilization of a methanol-d_4 solution of saframycin A was repeated until the NH signal disappeared. The sample concentration was *ca.* 0.5% (w/v) and a small amount of tetramethylsilane (TMS) was added as an internal standard.

^{1}H-NMR Measurements——^{1}H-NMR measurements were performed at 25±0.5 °C on a 400 MHz, JEOL GX-400 spectrometer in the Fourier transform mode. Generally, free induction decay (FID) was accumulated by using 16K data points for a spectral width of 4000 Hz.

T_1 measurements were made by the inversion recovery method utilizing different 15 t values with a pulse delay of more than 10 times the longest T_1 value. T_1 values were obtained by an exponential fit of the initial part of the experimental curves of magnetization recovery. The NOE difference spectra were obtained by using 8K data points. The irradiation time and pulse interval were greater than 5 times the longest T_1 value.

Computational Method

Spin-Lattice Relaxation Times——Equations describing the spin-lattice relaxation behavior of an n-spin system have been established by Noggle and Schirmer.[12] If all protons exist on a rigid molecule and relax dominantly by intramolecular dipolar interactions, the following equations hold.

$$-\frac{dI_i(t)}{dt}=\left(\sum_{j\neq i}\rho_{ij}\right)(I_i(t)-I_0)+\sum_{j\neq i}\sigma_{ij}(I_j(t)-I_0) \tag{1}$$

where

$$\rho_{ij}=\frac{\gamma_H^4\hbar^2}{r_{ij}^6}\left(\frac{1}{10}\cdot\tau_c+\frac{3}{10}\cdot\frac{\tau_c}{1+\omega_H^2\tau_c^2}+\frac{6}{10}\cdot\frac{\tau_c}{1+4\omega_H^2\tau_c^2}\right)$$

is the intramolecular direct dipole–dipole relaxation, and

$$\sigma_{ij}=\frac{\gamma_H^4\hbar^2}{r_{ij}^6}\left(-\frac{1}{10}\cdot\tau_c+\frac{6}{10}\cdot\frac{\tau_c}{1+4\omega_H^2\tau_c^2}\right)$$

is the intramolecular cross relaxation between protons i and j. $I_i(t)$ and $I_j(t)$ are the longitudinal magnetizations of protons i and j at time t after applying a 180° pulse at $t=0.0$ s. I_0 is the thermal equilibrium magnetization, τ_c the correlation time for molecular motion, r_{ij} the distance between protons i and j, and γ_H and $\hbar$ have their usual physical meanings. Equation 1 was solved numerically by the following procedure and T_1 values were obtained from the simulated relaxation curves by the non-linear least-squares treatments as described above.

In the case of internuclear distances between methyl and other protons, effective values were given to include averaging effects due to the rapid internal rotation of the methyl groups. Assuming isotropic overall tumbling motion, the same τ_c value was assigned to all but the methyl protons, to which an effective value τ_c^{eff} was assigned separately for the above reason. These motional parameters were determined as the values giving a minimum in the quantity as defined by Eq. 2.

$$q=\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left[\left(\frac{1}{T_{1,i}^{obs}}-\frac{1}{T_{1,i}^{calcd}}\right)\Big/\frac{1}{T_{1,i}^{obs}}\right]^2} \tag{2}$$

Steady-State NOE——Steady-state NOE was calculated from Eq. 3,

$$\left(\sum_{j\neq i}\rho_{ij}\right)\eta(i,k)+\sum_{j\neq i}\sigma_{ij}\eta(j,k)=\sigma_{ik} \qquad (i\neq k) \tag{3}$$

where ρ_{ij} and σ_{ij} have the same meanings as in Eq. 1, and $\eta(i,k)$ is the steady-state nuclear Overhauser enhancement factor of nucleus i on irradiation of nucleus k. For the quantities τ_c and internuclear distances in Eq. 3, the values optimized in the process of T_1 calculation were substituted.

Calculation of these relaxation parameters as well as the coordinates of model conformations, which were derived from the X-ray result on saframycin C[13] and refined by Crippen's distance geometry approach,[14] was performed by the RSCA program. Details of the RSCA program will be presented elsewhere.

Distance Geometry Approach——The problem treated by this method is to calculate atomic coordinates for a molecule which are consistent with the constraints given in terms of upper and lower bounds on the interatomic distances between n atoms (n is the total number of atoms in a molecule). Usually these bounds are determined by various experimental methods, and in this work the interatomic distances derived from T_1 and NOE data were used to restrict the conformation of saframycin A.

A qualitative outline of the method will be given below; for details of the computation, refer to the literature.[14]

(i) Selection of Bounds: Non-bonded distances estimated by NOE and T_1 measurement were used to set the bounds. Other upper and lower bounds were determined by the interatomic distances obtained from the X-ray result on saframycin C, with the exception of several bond lengths for which the reported values seemed to be unusual; in the latter cases, the standard values were applied.

(ii) Determination of the Initial Coordinates: After the violations of the inequalities (Eq. 2.20 and 2.21 of ref. 14*a*) were corrected according to the procedure of Braun *et al.*,[14b] the matrix of the distances **D** which is consistent with the requirements of the bounds was calculated. Next the metric matrix **G** was calculated from the distance matrix **D** (Eq. 24 and 25 of ref. 14*b*). **G** is a real symmetric matrix, by definition, and therefore can be solved to obtain its eigenvalues and eigenvectors. A trial set of atomic coordinates can be computed from the first three largest eigenvalues and their corresponding eigenvectors.

(iii) Distance Refinement: Because of the drastic simplification, the computed atomic coordinates usually violate the given distance constraints. To bring back the coordinates within the distance constraints, the penalty function defined by Eq. 4 is minimized.

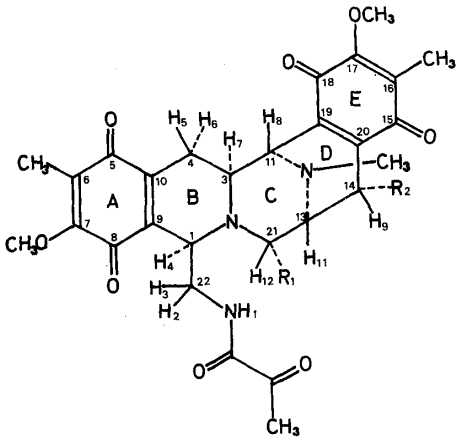
$$F=\sum_{i<j}{}'(d_{ij}^2-u_{ij}^2)^2+\sum_{i<j}{}'(d_{ij}^2-l_{ij}^2)^2 \tag{4}$$

where the summation of the first and second terms is made only for the distances outside the given upper and lower bounds respectively.

Results and Discussion

Assignments

Figure 1(a) shows the 400 MHz, ^{1}H-NMR spectrum of saframycin A in C_6D_6. All signals except for that of H_3 are resolved completely. The chemical shifts and coupling constants are summarized in Table I. The assignments were made by decoupling and NOE difference spectra techniques, and the previous assignments[7] were confirmed except for the geminal proton pairs H_2, H_3, and H_9, H_{10}. The previous assignments to H_9 and H_{10} were reversed in



saframycin A: $R_1=CN$, $R_2=H_{10}$
saframycin C: $R_1=H$, $R_2=OCH_3$

Chart 1. Structural Formulae of Saframycin A and C

TABLE I. Proton Chemical Shifts and Coupling Constants for Saframycin A in C_6D_6

Proton	Chemical shift (δ)	Coupling constants (Hz)
H_1	6.33	$J_{1,2}=3.7$, $J_{1,3}=9.5$
H_2	2.50	$J_{2,1}=3.7$, $J_{2,3}=14.2$, $J_{2,4}=3.7$
H_3	3.76	$J_{3,1}=9.5$, $J_{3,2}=14.2$, $J_{3,4}=1.5$
H_4	3.87	$J_{4,2}=3.7$, $J_{4,3}=1.5$, $J_{4,5}=2.7$
H_5	1.32	$J_{5,4}=2.7$, $J_{5,6}=17.6$, $J_{5,7}=11.4$
H_6	2.89	$J_{6,5}=17.6$, $J_{6,7}=2.9$
H_7	3.02	$J_{7,5}=11.4$, $J_{7,6}=2.9$, $J_{7,8}=2.9$
H_8	3.90	$J_{8,7}=2.9$, $J_{8,11}=2.5$
H_9	1.68	$J_{9,10}=21.0$, $J_{9,11}=0.5$
H_{10}	2.33	$J_{10,9}=21.0$, $J_{10,11}=7.3$
H_{11}	2.55	$J_{11,9}=0.5$, $J_{11,10}=7.3$, $J_{11,12}=2.4$
H_{12}	2.90	$J_{12,11}=2.4$

Chemical shifts are given relative to internal TMS as a reference.

this study and the assignments to H_2 and H_3 could be settled based on the conformation of the pyruvamide side chain derived from the present relaxation studies (see below).

The coupling constant between H_{11} and H_{12} is 2.4 Hz for saframycin A and the corresponding coupling constants between H_{11} and methylene protons on C_{21} are 2.0 and 3.0 Hz for saframycin C (see Chart 1). This indicates that the H_{11}–C_{13} bond nearly bisects the angle H_{12}–C_{21}–CN and that coupling constants cannot distinguish the two possible orientations of the nitrile group on C_{21}.

Nuclear Overhauser Effects

From the Dreiding model of saframycin A, H_{12} was expected to be proximate to H_9, H_{11}, H_4, and probably the methylene protons of the pyruvamide side chain for the epimer with the α-axial configuration of the CN group at C_{21}, which will be abbreviated to E-A hereinafter, while H_{12} should be proximate to H_7, H_{11}, and H_4 for the epimer with the β-equatorial configuration of the CN group at C_{21}, which will be called E-B. Thus NOE measurements were expected to differentiate these two possible epimers.

Since the two components of the H_7 signal are separated by *ca.* 11 Hz, a sufficiently strong irradiation power to saturate both components caused the adjacent signals, H_6 and H_{12}, to saturate simultaneously. To avoid this difficulty, Neuhaus' method[15] was adopted.

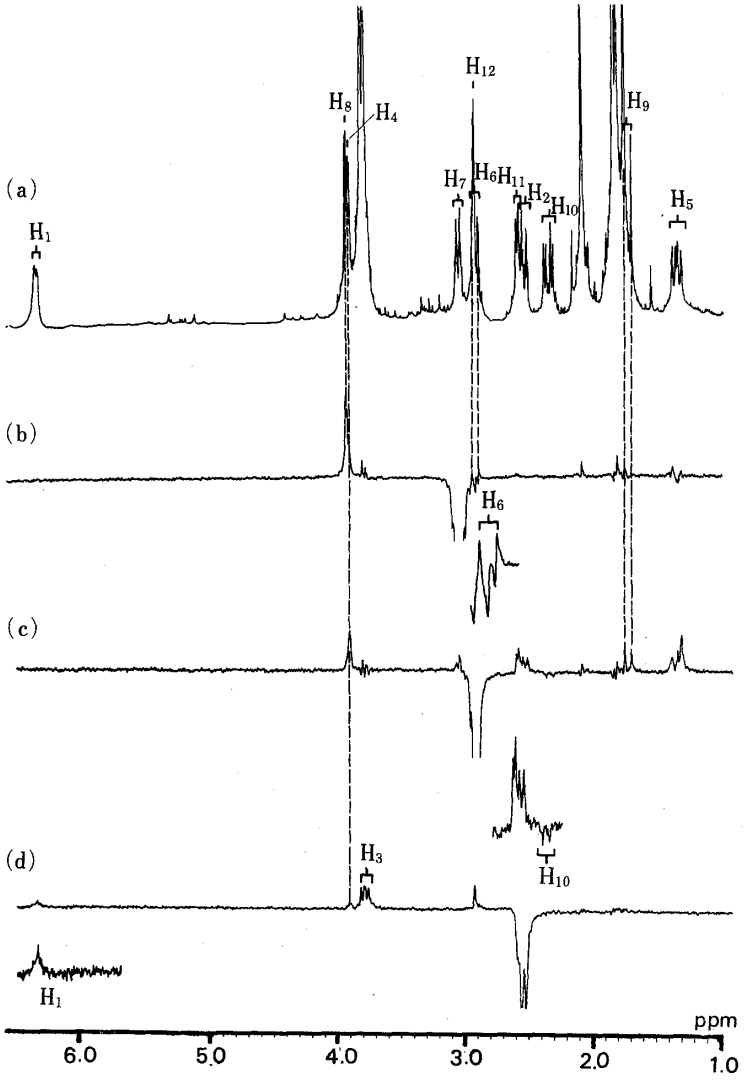


Fig. 1. 400 MHz ^{1}H-NMR Spectra for Saframycin A in C_6D_6

(a), Normal spectrum; (b), (c), and (d), H_7, H_{12}, and H_2 were irradiated respectively. In (b) and (d), Neuhaus' method was adopted for obtaining high selectivity.

Each component of the signals of H_7 was irradiated separately and the two resulting spectra were added later to cancel out the individual SPT (selective population transfer) effects and to maintain high selectivity (Fig. 1(b)). A large NOE at H_8 and a small NOE at H_6 are observed on irradiation of H_7, but no enhancement is observed at H_{12}. In contrast, H_{12} shows negative intensity but this cannot be taken as negative NOE, because in either E-A or E-B, H_7, H_{12}, and any other proton of the molecule cannot constitute a three-spin system with a relation of the interatomic distances, $r_{AX} > r_{AM}$, r_{MX}, which is necessary for a negative NOE to arise.[12] The apparent negative signal may be ascribed to partial saturation of H_{12} by irradiation of H_7.

Irradiation of H_{12} inevitably accompanies saturation of H_6 because of signal overlapping. Apparent signal enhancements of H_7 and H_5 seen in Fig. 1(c) should result from the saturation of H_6. In Fig. 1(c), NOE is observed at 1.68 ppm ($\eta = 0.05$) and 2.31 ppm ($\eta = -0.03$), which were previously assigned to H_{10} and H_9, respectively. In the epimer E-A, H_{12}, H_9, and H_{10} meet the conditions of a three-spin system with $r_{12,10} > r_{12,9}$, $r_{9,10}$. Therefore a negative NOE is expected for H_{10} rather than H_9 for E-A, while no NOE is expected for either H_9 or H_{10} for E-B when H_{12} is saturated. This indicates that the CN group

TABLE II. NOE for Saframycin A

Irradiated	Enhanced	η_{obs}[a]	η_{calcd}[a,b] (E-A)	(E-B)
H_7[c]	H_4	(0.00)[c]	0.03	0.02
	H_6	(0.01)[c]	0.05	0.05
	H_8	(0.11)[c]	0.23	0.23
	H_{12}	(0.00)[c]	0.00	0.07
H_{12}	H_2	0.06	0.07	0.00
	H_3	—[d]	−0.03	0.00
	H_4	0.07	0.11	0.12
	H_7	0.00[e]	0.00	0.06
	H_9	0.05	0.07	0.00
	H_{10}	−0.03	−0.03	−0.01
	H_{11}	0.07	0.11	0.14
H_2[c]	H_1	(0.07)[c]	0.06	0.06
	H_4	(0.02)[c]	0.08	0.09
	H_{12}	(0.09)[c]	0.14	0.00

a) NOE factor η was defined as $\eta=(I_{irr}-I_0)/I_0$, where I_0 and I_{irr} are peak intensities of blank and irradiated spectra, respectively. *b*) Geometrical and motional parameters similar to those for the T_1 calculation were used. *c*) To obtain high selectivity, Neuhaus' method was adopted. Thus, the NOE factors are reduced, because the signals are not completely saturated in these cases.[15] NOE calculation was done just for reference. *d*) Not observable due to signal overlapping. *e*) The apparent signal enhancement seen in Fig. 1(c) is ascribed to saturation of H_6.

at C_{21} takes α-axial orientation and the assignments of H_9 and H_{10} should be reversed.

Comparison of the coupling constants of saframycin A with those of saframycin C supports this revision. According to Lown *et al.*[7] $J_{11,9}$ of saframycin C in C_6D_6 is less than 0.5 Hz and corresponds to the observation from X-ray analysis that the dihedral angle H_{11}–C_{13}–C_{14}–H_9 was 78.8°. Since no drastic conformational change of ring D in saframycin A was detected as shown below, their assignments of coupling constants, *ca.* 0.5 Hz to $J_{11,10}$ and 7.5 Hz to $J_{11,9}$, conflict with the above discussion, and the assignments, *ca.* 0.5 Hz to $J_{11,9}$ and 7.5 Hz to $J_{11,10}$ seem more reasonable.

Another NOE is observed at 2.50 ppm. This signal was assigned to H_3 previously,[7] but it should be reassigned to H_2 on the basis of the discussion below about the pyruvamide side chain conformation. As shown in Fig. 1(d), a similar NOE can be seen in reverse; irradiation of H_2 leads to an enhancement of the signal of H_{12}.

These observations suggest that the configuration of saframycin A corresponds to the epimer E-A. Table II shows the observed NOE factors together with calculated ones for E-A and E-B. The fair agreement between NOE factors observed and calculated for E-A confirmed the interpretation described above semi-quantitatively.

Spin-Lattice Relaxation Time

(i) Configuration——As shown in the previous section, NOE difference spectra were found to be valuable for the problem of spectral assignment and discrimination of epimeric pairs, but it is difficult or impossible to measure them in the case of partial overlapping or close proximity of observed and irradiated signals. In such cases, T_1 measurements may serve as another tool for conformational and configurational studies.

Figure 2 shows the main part of the inversion recovery Fourier transform (IRFT) spectra of saframycin A in C_6D_6. Observed T_1 values are listed in Table III. Referring to the above section, we consider that H_{12} is surrounded by four protons (H_2, H_4, H_9, and H_{11}) within 3.0 Å for the epimer E-A, and three protons (H_4, H_7, and H_{11}) for the epimer E-B. Thus the T_1 value of H_{12} is expected to be configuration-sensitive and this proton should relax faster in E-

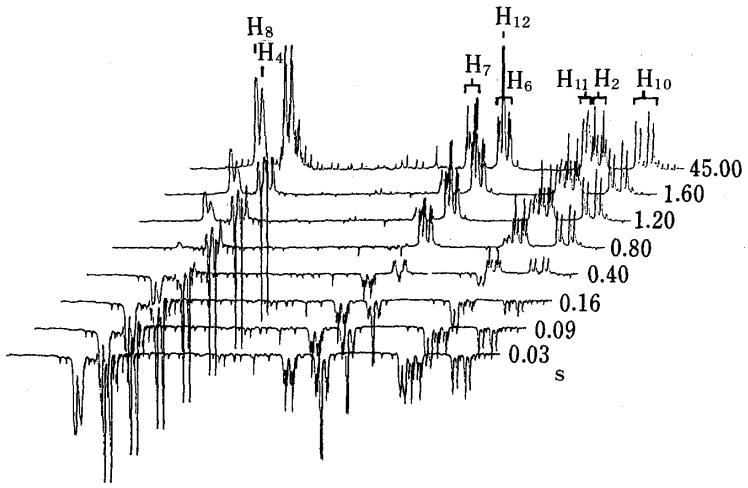


Fig. 2. Spectra Covering the Main Part of Saframycin A Obtained by Using the Inversion Recovery Sequence in C_6D_6

Fifteen different delay times from 0.02 to 1.60 s were applied (not all of them are shown).

TABLE III. T_1 Values for Saframycin A

Proton	Obs[a]	Calcd[b] (E-A)	Calcd[b] (E-B)
H_1	1.16±0.03	1.31	1.56
H_2	0.37±0.01	0.32	0.37
H_4	1.25	1.26	1.19
H_5	0.49±0.05 (0.57)[c]	0.33	0.35
H_6	0.44±0.02	0.37	0.37
H_7	1.27±0.08	1.41	1.21
H_8	1.13	1.22	1.21
H_9	0.36±0.05	0.35	0.41
H_{10}	0.39±0.03	0.30	0.30
H_{11}	0.89±0.09	0.86	0.78
H_{12}	0.67±0.03	0.76	1.30

a) Obs values are the averages of two or three experiments. Three-fold standard deviations of three experiments are given. *b*) The calculation was carried out by using motional parameters, $\tau_c = 0.84 \times 10^{-10}$ s, $\tau_c^{eff} = 0.15 \times 10^{-10}$ s and dihedral angles of the pyruvamide side chain, $\psi_1 = 60°$ and $\psi_2 = 90°$ for both epimeric models E-A and E-B. Ring conformation was also refined by the distance geometry approach. *c*) The value for the sample in which NH of the pyruvamide was replaced by ND.

A than in E-B. Comparison of T_1 values calculated for the two model configurations, E-A and E-B, with observed ones could determine which corresponds to the correct configuration. T_1 values given in the third and fourth columns of Table III are the optimal ones among conformational and rotational isomers examined for the epimeric models E-A and E-B, respectively. T_1 values of three methine protons (H_4, H_8, and H_{11}), which seem rather less sensitive to the configuration at C_{21}, are well reproduced for both epimers. This means that the geometric and motional parameters used are reasonable and that the calculated values are reliable. The calculated T_1 value of H_{12} is 0.76 s for E-A and 1.30 s for E-B, while the observed value was 0.67 s. This result clearly points to the α-axial configuration of the nitrile group at C_{21} (the epimer E-A).

In the present case, therefore, it was possible to discriminate the correct configuration of an epimer without direct comparison of T_1 values measured for both members of the epimeric

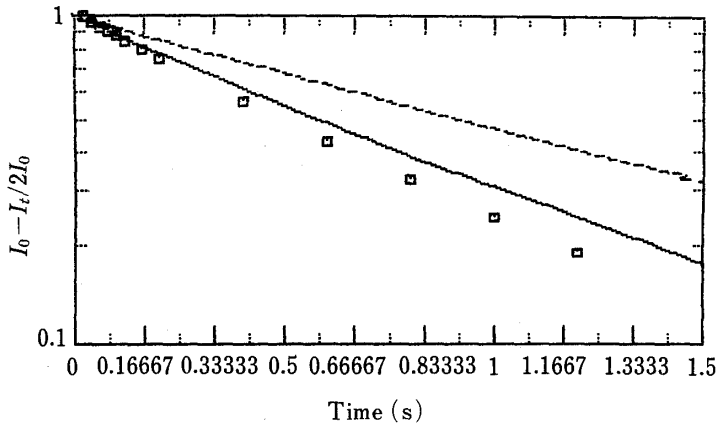


Fig. 3. Semi-logarithmic Plot of Magnetization Recovery of H_{12}
——, calculated for E-A; – – –, calculated for E-B; □, observed values.

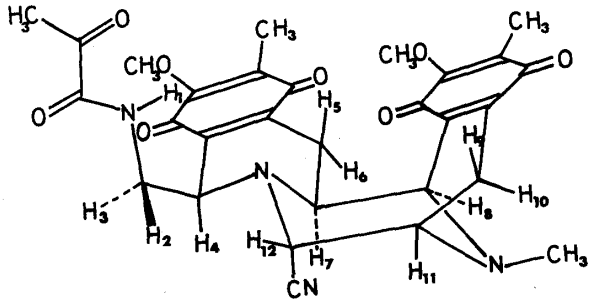


Fig. 4. The Average Conformation of Saframycin A in C_6D_6

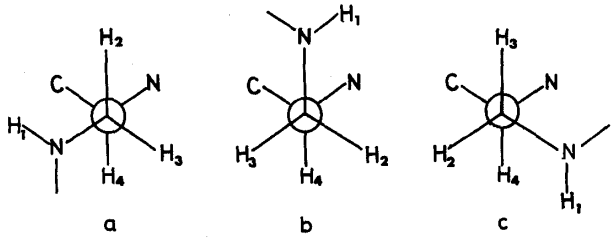


Chart 2

pair. Figure 3 shows the observed and calculated relaxation curves for H_{12}, demonstrating an excellent fit between the observed and the calculated relaxation curve for the α-axial epimer, E-A.

Taking account of the reaction mechanism proposed by Lown *et al.*[4] that DNA binding of saframycin A is initiated by the enzymatic reduction of its quinone moiety (ring A) with concomitant loss of the nitrile group, the α-axial configuration seems to be favorable to assist this initial step because the lone pair on N_2 is *trans* to the leaving nitrile group.

(ii) Pyruvamide Side Chain——The above discussion is valid insofar as no conformation possible to the epimer E-B can satisfy the observed T_1 values. It is, therefore, necessary to take into account the conformational flexibility of the molecule to rule out this possibility.

In the case when a flexible group such as the pyruvamide side chain is involved, it is useful to obtain directly the intramolecular distances between proton k in the flexible side chain and proton i in the rigid molecular frame, which can be obtained from an appropriate set of T_1 values for the parent compound and its D-substituted one, using Eq. 5.

$$\frac{1}{T_{1,i}(H_k)}-\frac{1}{T_{1,i}(D_k)}=0.937\cdot\frac{\gamma_H^4\hbar^2}{r_{ik}^6}\left(\frac{3}{10}\cdot\frac{\tau_c}{1+\omega_H^2\tau_c^2}+\frac{12}{10}\cdot\frac{\tau_c}{1+4\omega_H^2\tau_c^2}\right) \quad (5)$$

Replacement of the NH proton on the pyruvamide side chain by deuterium caused a significant increase of the T_1 value of H_5 alone, from 0.49 to 0.57 s. From Eq. 5, the distance, $r_{1,5}$, was estimated as 2.4 to 2.5 Å for τ_c values ranging from 0.6 to 0.9×10^{-10} s, reasonable for small organic molecules. This result, along with the NOE observed at H_2 on saturation of H_{12}, indicates that the average conformation of the pyruvamide side chain is as depicted in

Fig. 4. This result is consistent with a high population of the rotomer b around the C_1–C_{22} bond (Chart 2), which was suggested previously[7] from the vicinal coupling constants, $J_{2,4}=3.7$ Hz and $J_{3,4}=1.5$ Hz.

(iii) Model Refinement——Preliminary calculations of relaxation parameters of saframycin A were made by assuming a ring conformation similar to that of saframycin C for both E-A and E-B and the calculated values were compared with the observed ones. The results were not satisfactory from a quantitative point of view but are adequate to indicate that E-A corresponds to the correct epimer. However, there were several disagreements between observed and calculated values.

In order to attain a better quantitative agreement between the calculated and observed T_1 values, the conformation of the ring system was revised by using Crippen's distance geometry approach,[14] in which interatomic distances obtained from NOE and the deuterium substitution effect on T_1 value were used to set the bounds. The final constraints used and the resulting conformation are illustrated in Fig. 5.

Based on the revised ring conformation obtained above, where the pyruvamide side chain was fixed in the conformation depicted in Fig. 4, a number of calculations of T_1 values were carried out to search for the optimal τ_c and τ_c^{eff}. Two such calculations, carried out separately for each epimeric model, gave the same optimal values; $\tau_c=0.84\times10^{-10}$ s and $\tau_c^{eff}=0.14\times10^{-10}$ s. Since τ_c^{eff} was not sensitive to the T_1 values except for those of the methyl protons, more precise treatment of methyl rotation[16] was not made. Optimization of the pyruvamide side chain was carried out by changing two dihedral angles, H_2–C_{22}–C_1–H_4 (ψ_1) and H_1–N–C_{22}–H_2 (ψ_2), in steps of 30° from 0° to 360°. The T_1 values in Table III correspond to those of the optimal conformer. In the case of epimeric model E-B, a satisfactory agreement between observed and calculated T_1 values could not be found for any of the conformational and rotational isomers examined.

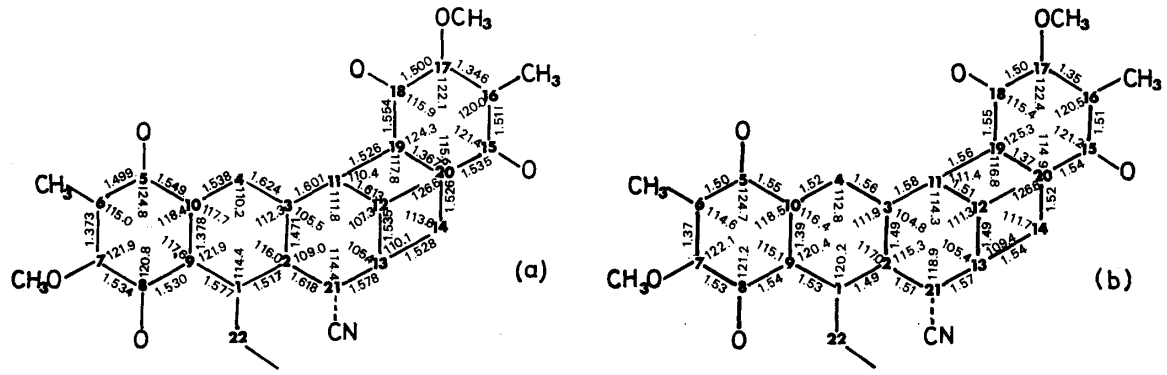


Fig. 5. Bond Lengths and Bond Angles for Saframycin C and Saframycin A

(a), Bond lengths and bond angles for saframycin C in crystals; (b) bond lengths and bond angles for saframycin A derived from the distance geometry approach.

In the application of the distance geometry approach, the following constraints were given.

i) H_1–H_5 was constrained by the upper and lower bounds of 2.5 Å and 2.4 Å, respectively, from the effect of deuterium substitution of H_1 on the T_1 value of H_5. H_{12}–H_2 was restricted in a range of 2.5 Å to 2.2 Å based on the NOE observable between H_2 and H_{12}.

ii) The upper and lower bounds of C_3–C_4 and C_3–C_{11} were determined as 1.56 Å and 1.53 Å, respectively, and 1.48 Å and 1.46 Å were used for N_{12}–C_{11}, N_{12}–C_{13}, and N_2–C_{21} as the upper and lower bounds to set these bond lengths within the range of the standard values of these bond types.

iii) The other bounds of the interatomic distances were derived from the X-ray analysis of saframycin C. To allow the movement of atoms in the computational process, the upper bound u_{ij} of the distance d_{ij} between atoms i and j was set as $u_{ij}=d_{ij}(1.0+f)$ and the lower bound l_{ij} as $l_{ij}=d_{ij}(1.0-f)$, where f was 0.001 for 1, 2 distances (bond lengths) and 1, 3 distances (i and j bonding to the same atoms), and 0.1 for other distances. The penalty function (Eq. 4) was converged to 0.081.

TABLE IV. Pople's Puckering Parameters for Saframycin C (by X-Ray Analysis) and the Final Model Conformation of Saframycin A

	Saframycin A	Saframycin C	
Ring B			
Q	0.444	0.509	Half-boat
φ	139.4	150.2	
θ	57.0	64.5	
Ring C			
Q	0.554	0.659	Chair
φ	275.4	291.4	
θ	11.9	4.3	
Ring D			
Q	0.513	0.553	Half-boat
φ	43.9	53.4	
θ	49.4	49.9	

For six-membered rings, puckering parameters can be expressed as a "spherical polar set" (Q, θ, φ), where Q is the total puckering amplitude and θ ($0° \leqq \theta \leqq 180°$) and φ ($0° \leqq \varphi \leqq 360°$) are angles specifying the positions on a sphere. The polar positions ($\theta = 0°$, or 180°) correspond to a pure chair conformation and the positions on the equator ($\theta = 90°$) with $\varphi = 0°$, 60°, 120°, 180°, 240°, and 300° correspond to pure boat conformations. Other commonly observed conformations can be located on this coordinate system. See reference 17 for details.

(iv) Ring Conformation——For comparison of the conformations, the bond lengths, bond angles, and Pople's general puckering coordinates[17] in the final conformation of saframycin A and those for saframycin C in crystals (see Fig. 5 and Table IV) were calculated. From Pople's parameters, the conformations of rings B, C, and D of saframycin A are found to be similar to those of saframycin C, though there are small but not negligible differences. In the final model, ring B deforms in the direction from a half boat to a half chair and the chair conformation of ring C is slightly twisted. Since the energy aspect was neglected, we cannot discuss these differences quantitatively. However, this may be qualitatively correct, because the conformation of ring B, a little deformed in the direction from a half boat to a half chair (which explains the observed NOE), could be realized in the Dreiding model.

The detection of small conformational differences between saframycin A in solution and saframycin C in crystals suggests that the quantitative treatment of T_1 values and NOE factors in combination with the distance geometry approach is an effective tool to elucidate the conformation of the molecule in solution semi-quantitatively. Further study in this direction is now being undertaken.

Conclusion

The α-axial configuration of the nitrile group of saframycin A and the conformation of saframycin A in C_6D_6 were determined by means of quantitative treatments of relaxation parameters. The relaxation study also made it possible to assign correctly two geminal proton pairs for which the previous assignments were ambiguous.

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