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THE CONFORMATION OF RIFAMYCIN S IN SOLUTION BY ^{1}H NMR SPECTROSCOPY

G. G. GALLO,* E. MARTINELLI, V. PAGANI and P. SENSI
Laboratori Ricerche Lepetit S.p.A., 20158 Milano, Italy

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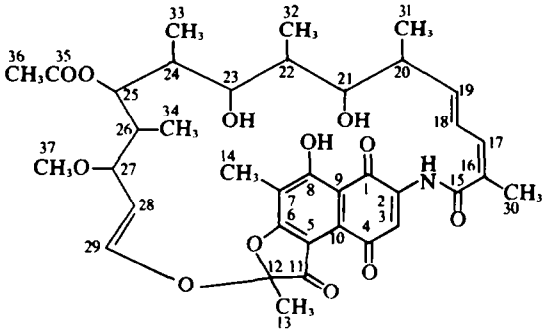
Abstract—The ^{1}H NMR spectra of rifamycin S in different solvents and at different temperatures strongly suggest the existence of a dominant conformer. The nine dihedral angles of the ansa chain from C-28 to C-19, considered conformationally flexible, were obtained from the vicinal interproton coupling constants by the Karplus equation and the proper alternative for each of them compares well with the corresponding values given by X-ray analysis in solid state.

All the possible conformations derivable from the intrinsic alternatives of the NMR method were calculated for the ansa chain between C-28 and C-19 and, by applying geometrical considerations, such as closure of the polygonal path of the ansa and the steric incompatibility between the various atoms of the ansa and of the chromophore, only two of them appeared real. Thus, NMR spectroscopy can be used for studying the conformation of the ansa chain of rifamycins in solution.

The rifamycins are a family of antibiotics obtained by fermentation and chemical modification.[1,2] They have been used successfully in the therapy of tubercular infections and of diseases caused by Gram-positive and Gram-negative bacteria.[3] These antibiotics have been shown to be inhibitors of the transcribing enzymes.[4] Their structure was elucidated by chemical degradation studies[5-7] and by X-ray analysis.[8] The structure of refamycin S, as



representative, consists of a naphthoquinone chromophore which is spanned by an aliphatic bridge, called the ansa chain. The spatial arrangement is visualized by the molecular model build up according to recent X-ray data,[9] shown in Fig 1.

From previous structure-activity relationship studies[2] it was inferred that the essential feature for the activity of rifamycins is the presence of the three free hydroxyls on C-21, C-23 and C-8 and of an oxygen function on C-1. Furthermore, it is thought that probably only a given conformation of the ansa chain allows the binding between the rifamycins and the target enzyme, which is the bacterial DNA dependent RNA polymerase. Thus, a definite spatial relationship has to exist among the mentioned oxygen functions[4] and this can be visualized by inspecting the molecular model. As the conformation of a molecule in the crystal state given by X-rays does not necessarily indicate the conformation in solution, particularly with a potentially flexible structure such as the large ansa chain of rifamycins, we undertook the study of the conformation of this biologically important molecule by ^{1}H NMR spectroscopy, taking advantage of the fact that most of the carbon atoms of the ansa chain carry hydrogens.

The spectrum of rifamycin S at 270 MHz in $CDCl_3$ is shown in Fig 2. On the basis of the chemical shift values and by complete decoupling experiments performed at 100 MHz by us and by other groups[5,7,10-13] all the 45 protons of the molecule had been previously assigned. With respect to chemical shifts, some interesting stereochemical aspects can be considered: (1) Two of the Me groups of the ansa chain appear unusually shielded; this fact, was the first indication for Prelog[14] that rifamycins were ansa compounds. In particular, CH_3-34 is most affected by the ring current of the aromatic chromophore and this fact is indicative that it is located above it.[15] (2) The chemical shifts of the protons H-17, H-18 and H-19 in the linear conjugated dienone system from C-15 to C-19 do not correspond to the expected electron density at these protons,[16] but are influenced by the anisotropic effect of the carbonyl group, i.e. H-17 and H-18 are deshielded: this fact has been observed in similar systems[17] and is consistent with the 16–17 *cis* and 18–19 *trans* configurations and with the 17–18 transoid conformation.

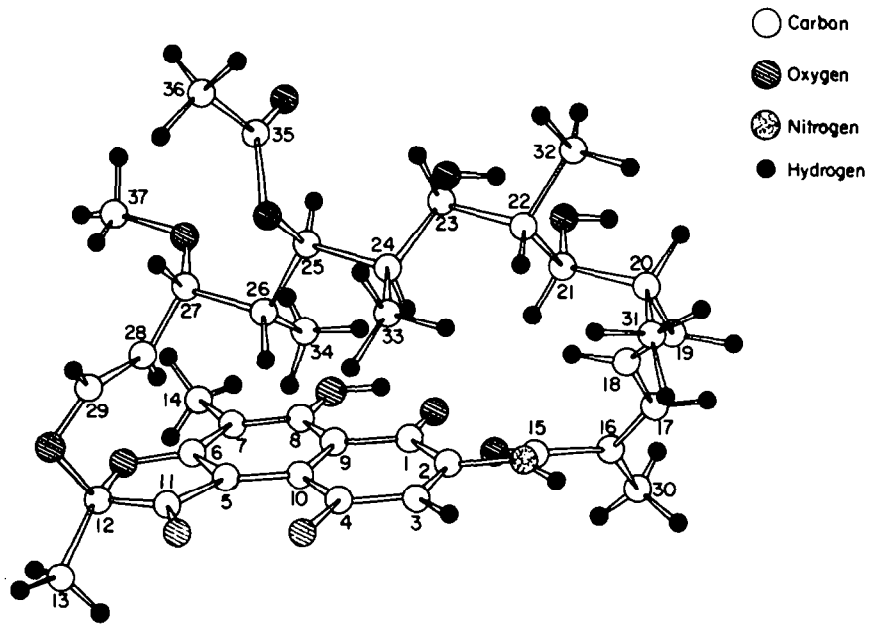


Fig 1. Stereomodel of rifamycin S according to X-rays.

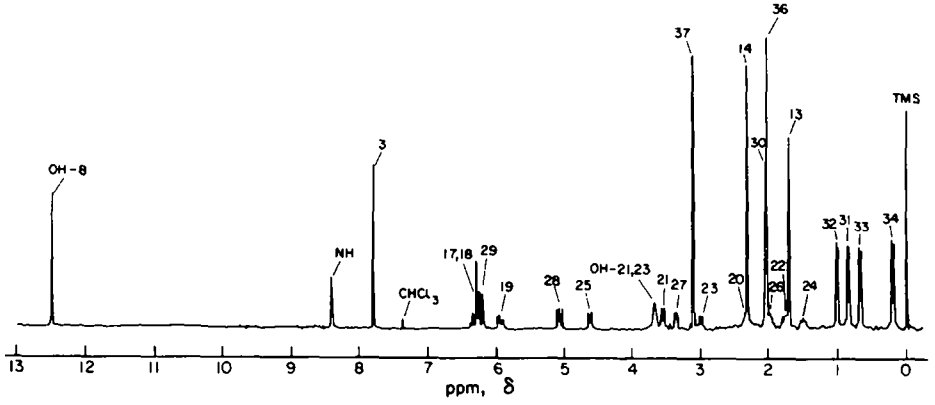


Fig. 2. ^{1}H NMR FT spectrum at 270 MHz of rifamycin S in $CDCl_3$.

Rifamycin S was examined at 100 MHz, in pyridine, methanol and dimethylsulphoxide in addition to chloroform. The spectra were fully interpreted and the chemical shifts values are reported in Table 1. Consideration of the solvent effect on the chemical shifts of the various protons does not concern the present work and will be reported elsewhere. The vicinal interproton coupling constants in the four different solvents are reported in Table 2. By comparing the values for each coupling constant in the four solvents it can be observed that they are about the same. For flexible systems it has been shown that the population of conformers in equilibrium at a given temperature is dependent on the solvent, as reflected by changes in the time-averaged coupling constants.[18] The absence of such an effect in rifamycin S can be considered as an indication of the existence of a dominant conformer. As a further test of conformational homogeneity, the spectra of rifamycin S were examined over a temperature range from −80° to 70°. Methanol-d4 was the most suitable solvent for low temperature whereas chloroform-d1 was used for high temperature. No change in coupling constants was observed from −80° to 70°.

The values of the observed coupling constants were used to determine the approximate dihedral angles between vicinal protons using the Karplus relationship.[19] According to this equation, for each J_{vic} four values of the angle are possible: the angle, its supplementary and the corresponding opposite angles. Because of the uncertainties introduced by

Table 1. Chemical shifts of rifamycin S in $CDCl_3$, C_5D_5N, CD_3OD and CD_3SOCD_3 solutions.[a]

Proton	$CDCl_3$	C_5D_5N	CD_3OD	CD_3SOCD_3
NH	8·38	9·70	*c*	9·54
H-3	7·77	8·18	7·66	7·32
OH-8	12·51	*c*	*c*	12·6
H-13	1·70	1·80	1·69	1·66
H-14	2·30	2·24	2·31	2·19
H-17	*b*	6·19	*b*	*b*
H-18	*b*	6·60	*b*	*b*
H-19	5·90	5·93	*b*	*b*
H-20	2·30	2·42	2·30	2·15
H-21	3·52	3·89	3·69	3·51
OH-21	3·62	*c*	*c*	4·32
H-22	1·76	1·83	1·75	1·70
H-23	2·97	3·40	3·09	2·90
OH-23	3·62	*c*	*c*	4·32
H-24	1·50	1·66	1·50	1·40
H-25	4·59	5·27	4·94	5·01
H-26	1·91	2·04	1·80	1·50
H-27	3·33	3·41	3·38	3·27
H-28	5·02	5·66	5·27	5·08
H-29	6·16	6·50	6·20	6·13
H-30	2·01	1·98	1·95	1·98
H-31	0·84	0·87	0·87	0·79
H-32	1·00	1·07	0·99	0·84
H-33	0·66	0·76	0·69	0·58
H-34	0·21	0·33	0·12	0·15
H-36	2·01	1·93	2·03	1·92
H-37	3·08	3·03	3·08	2·98

[a] In ppm downfield from tetramethylsilane as internal reference.
[b] Not determined.
[c] Not observable separately from the other mobile protons.

other structural factors, such as electro-negativity and orientation of substituents, hybridization of carbon atoms etc., for obtaining J_0 and J_{180} values for each ethane segment of the ansa chain, a value of J = 10 Hz was assumed as a reasonable approximation, taking into consideration the various data reported in the literature.[20] The dihedral angles so

Table 2. Vicinal interproton coupling constants (J_{vic}, Hz) of rifamycin S in $CDCl_3$, C_5D_5N, CD_3OD and CD_3SOCD_3 solutions at 30°

	$CDCl_3$	C_5D_5N	CD_3OD	CD_3SOCD_3
$J_{17,18}$	*a*	9·5	*a*	*a*
$J_{18,19}$	13·5	15·0	*a*	*a*
$J_{19,20}$	7·0	7·0	*a*	*a*
$J_{20,21}$	9·5	9·0	10·0	8·5
$J_{21,22}$	0·5	1·0	1·5	2·0
$J_{22,23}$	1·5	1·5	2·0	2·0
$J_{23,24}$	10·0	9·5	9·0	10·0
$J_{24,25}$	1·0	1·0	1·5	2·0
$J_{25,26}$	10·5	10·0	10·5	10·0
$J_{26,27}$	2·5	2·5	3·0	3·0
$J_{27,28}$	8·0	8·5	8·0	8·0
$J_{28,29}$	12·5	13·0	12·5	12·5
$J_{31,20}$	7·0	7·0	7·0	7·0
$J_{32,22}$	7·0	7·0	7·0	7·0
$J_{33,24}$	7·0	7·0	7·0	7·0
$J_{34,26}$	7·0	7·0	7·0	7·0

[a] Not determined.

calculated from C-28 to C-19 are reported in Table 3. For seven of these angles all the four possible alternatives are shown, while for two of them only two alternatives are intrinsically possible. Thus, the ansa chain has only been considered as conformationally flexible from C-28 to C-19, while the other part has been taken as fixed as a consequence of the conjugation operating in the enol system at one side and in the dienamide system at the other side (Fig 1).

The successive step was the comparison of these values with those obtainable from the X-ray analysis, which was performed on crystals of the p-iodo-anilide of rifamycin B[8] and which was recently refined.[9] As X-rays give the coordinates only of C, O and N atoms and not of H atoms, for deriving the nine dihedral angles between protons the following mathematical approach was used. The ansa chain was split into nine segments, each one made of four

Table 3. Comparison between the dihedral angle values as obtained by ^1H NMR and X-rays

No	Dihedral angle	J_{vic} H/H[a]	^1H NMR values according to Karplus equation	X-ray value calculated from the torsional angle between carbon atoms
1	H-28/H-27	8·1	±23° and ±157°	+177°
2	H-27/H-26	2·8	±56° and ±124°	+70°
3	H-26/H-25	10·0	0° and 180°	+174°
4	H-25/H-24	1·4	±66° and ±114°	−85°
5	H-24/H-23	10·0	0° and 180°	+174°
6	H-23/H-22	1·8	±63° and ±117°	+53°
7	H-22/H-21	1·2	±67° and ±113°	+61°
8	H-21/H-20	9·2	±12° and ±168°	+170°
9	H-20/H-19	7·0	±31° and ±149°	+49°

[a] Averaged value from Table 2.

C atoms and of the two internal vicinal protons. The Newman projection of one of these segments containing C-27, C-26, C-25, C-24 and H-26, H-25 is shown in Fig 3. Let α be the value of the dihedral angle between the carbons C-27 and C-24 as measured by X-rays; let α_1 and α_2 be the theoretical values of the angles between C-27, H-26 and H-25, C-24, respectively, projected on the plane perpendicular to the central bond C-26, C-25; let α_3 be the dihedral angle between the protons H-26, H-25. The numerical value of α_3 can be obtained by the equation: $\alpha = \alpha_1 + \alpha_2 + \alpha_3$. All the nine dihedral angles concerned were calculated in this way starting from X-ray data and they are also reported in Table 3 in comparison with the values obtained by ^{1}H NMR. It can be derived that the absolute magnitude of the dihedral angles as calculated by the two methods, ^{1}H NMR in solution and X-ray in solid state, are in qualitative agreement, if one considers the proper NMR alternative.

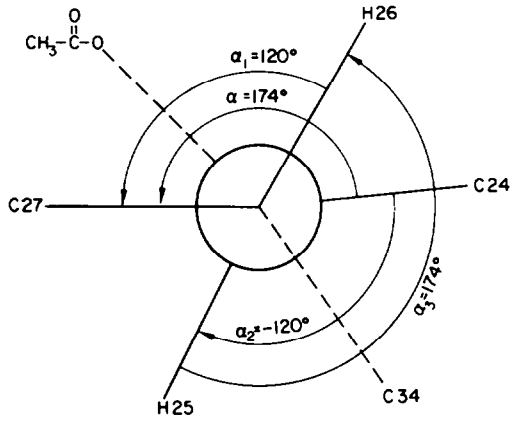


Fig 3. Newman projection of the segment C-27, C-26, C-25, C-24 of the ansa chain. The angle is taken as positive when the lower numbered atom eclipses the higher numbered one counterclockwisely, by going from C-28 to C-19.

At this point it became apparent that the NMR method alone could be more informative about the conformation of rifamycins in solution, because not all the NMR alternatives given by the Karplus equation would be able to bring about the closure of the polygonal path from C-28 to C-19. In other words, it was considered worthwhile to investigate which combinations of the NMR alternatives would yield the closure of the ansa. Accordingly, for the nine segments of the ansa chain concerned the following procedure, Fig 3, using the third segment as example was applied. As the dihedral angle between H-26 and H-25 (α_3) takes on the four values given by the Karplus equation (Table 3), four values of the dihedral angle between C-27 and C-24 (α) can be obtained and, consequently, four different positions of the last carbon atom (C-24) can be obtained. The coordinates of C-24 were obtained from the coordinates of C-26 by the equations:

$$x_{24} = d_1 \cos\alpha \cos\gamma + x_{26}$$
$$y_{24} = d_1 \sin\alpha \cos\gamma + y_{26}$$
$$z_{24} = d_1 \cos\beta + d_2 + z_{26}$$

where d_1 is the distance between C-25 and C-24, d_2 is the distance between C-26 and C-25, β is the angle between $\overrightarrow{C\text{-}26\text{–}C\text{-}25}$ and $\overrightarrow{C\text{-}25\text{–}C\text{-}24}$, γ is $\pi/2 - \beta$. Obviously, d_1, d_2, β and γ correspond to the X-ray data, while α_3 and consequently α correspond to NMR data. When also α corresponds to X-ray data the polygonal path closes at a distance of 0·002 Å. Calculations are simplified by a transformation of the coordinate system for each segment of the ansa. For the third segment the new system of coordinates has C-26 as the origin, $\overrightarrow{C\text{-}26\text{–}C\text{-}25}$ as the Z-axis, $\overrightarrow{C\text{-}27'\text{–}C\text{-}26}$ as the X-axis and the vector ZAX as the Y-axis, where C-27′ is the projection of C-27 on the plane perpendicular to the central bond $\overrightarrow{C\text{-}26\text{–}C\text{-}25}$.

The repetition of this procedure for all the 9 segments generates $4^7 \times 2^2 = 65{\cdot}536$ different conformations of the ansa chain that are possible according to the NMR data. The whole procedure was carried out twice by moving along the ansa chain in opposite directions, i.e. from C-28 to C-19 and vice-versa. Some conformations were eventually considered as real alternatives depending on the distance between the calculated end point, i.e. C-19′ (or C-28′) and the corresponding point as determined by X-ray, i.e. C-19 (or C-28). Thus, values of those distances not greater than twice the values obtained with the NMR series corresponding to X-rays, which was taken as reference, were accepted. After this procedure was performed, only five solutions were left, which are reported in Table 4 together with the reference conformation. Also the distance between C-19′ and C-19 and that between C-28′ and C-28 for the six possible conformations are given. The figures in squares correspond to angles different from those of the reference series.

The five possible alternatives were checked, by the described procedure, if they were compatible with the geometry of the whole molecule, i.e., the distances between each carbon atom of the ansa chain and each atom of the aromatic chromophore were calculated. After this operation was performed the alternatives 4, 5 and 6 could be ruled out as unreal because of steric hindrance between some of the metioned C atoms. In particular, for alternative 4 the distance C-18 ··· N is 1·66 Å, for 5 C-21 ... O-1 = 1·89 Å and for 6 C-23 ··· C-9 = 1·26 Å. Of the two remaining conformations number 2 differs from the reference only in the dihedral angle H-21/H-20 and it cannot be considered a different alternative because of the small difference between ±168° and 180°. Thus, only

Table 4. Dihedral angles between protons and distances (Å) of calculated end-points (C-19′ or C-28′) from X-ray end-points (C-19 or C-28)

		Possible conformations					
N°	Dihedral angle	1 (Reference)	2	3	4	5	6
1	H-28/H-27	157°	157°	157°	−157°	−157°	−157°
2	H-27/H-26	56°	56°	56°	−56°	−56°	−124°
3	H-26/H-25	180°	180°	180°	180°	180°	180°
4	H-25/H-24	−66°	−66°	−114°	66°	−114°	−66°
5	H-24/H-23	180°	180°	180°	180°	180°	180°
6	H-23/H-22	63°	63°	117°	−63°	63°	63°
7	H-22/H-21	67°	67°	67°	113°	113°	113°
8	H-21/H-20	168°	−168°	168°	168°	168°	168°
9	H-20/H-19	31°	31°	31°	31°	31°	31°
DISTANCE C-19′ · C-19 BY C-28→C-19		0·75	1·04	0·96	1·44	1·47	1·18
DISTANCE C-28′ · C-28 BY C-19→C-28		1·71	2·75	1·76	1·17	1·05	1·80

number 3 must be considered a real NMR alternative conformation. The closure of the polygonal path in this case can be understood by considering that the differences in the dihedral angles H-25/H-24 and H-23/H-22 compensate each other.

In conclusion, by elaborating the ^{1}H NMR spectroscopy data according to the Karplus equation and by considering steric factors of the molecule, such as the closure of the ansa chain between C-28 and C-19 and the steric incompatibility between the carbon atoms of the ansa and of the chromophore, only two conformations appear possible in solution for the ansa chain. The first corresponds well to that obtained in solid state by the X-ray diffraction method; the second one differs from the former in two dihedral angles, leaving practically unchanged the mutual position of the two hydroxyl groups at C-23 and C-21, which are considered an essential feature for the activity of rifamycins. Thus, NMR spectroscopy can be used as a method for studying the conformation of the ansa chain of rifamycins in solution.

EXPERIMENTAL

Instrumentation. NMR spectra were recorded at 100 MHz on a Varian Associates XL-100 spectrometer and at 270 MHz on a Bruker WH-270 NMR spectrometer. The spin decoupling experiments were performed on the XL-100 instrument using the proper "gyrocode decoupler" frequency and keeping an internal deuterium lock on the peak of the various solvents employed. The spectra at different temperatures were performed by using a V-6040 variable temperature controller previously calibrated for high and low temperature ranges.[21]

Materials. A highly purified sample of rifamycin S was kindly supplied by Dr. R. Cricchio. The fully deuterated solvents methanol, chloroform and pyridine were purchased from Ciba and DMSO-d6 from Merck and were used without further purification.

Computing technique. Suitable Fortran IV (H) programs were set up and calculations were performed on IBM 360/65 and IBM 360/50 system.

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