Comparative Study of the Conformations of Rifamycins in Solution and in the Solid State by Proton Nuclear Magnetic Resonance and X-rays[1]

Luciano Cellai, Silvio Cerrini, and Annalaura Segre*

Istituto di Strutturistica Chimica "Giordano Giacomello", CNR, CP 10, 00016 Monterotondo Stazione, Rome, Italy

Mario Brufani

Gruppo di Chimica Biologica e Strutturistica Chimica, University of Rome, 00100 Rome, Italy

Walter Fedeli

Istituto di Chimica Farmaceutica e Tossicologica, University of Bologna, 40126 Bologna, Italy

Alessandro Vaciago

Chemical Crystallography Laboratory, University of Oxford, Oxford OX1 3PD, England

Received December 16, 1981

3-Substituted rifamycins S and SV have been studied by ^{1}H NMR in organic solvents and in D_2O, and the conformations in solution have been compared with those obtained in the solid state. Rifamycins exhibit four different isomers generated by the combination of two rotations, one relative to the amidic plane and the other relative to the plane containing the double bond C(28)=C(29). However, the conformation of the central part of the *ansa* chain and the spatial relationship between the four oxygens O-1, O-2, O-9, and O-10, essential for the antibacterial activity of these compounds, always remain the same, independent of the oxidation state at O-1, the kind of 3-substituent, whether the solid or solution state is involved, and the nature of the solvent.

Rifamycins constitute a well-known class of antibiotics whose action is explicated through the inhibition of bacterial DNA-dependent RNA polymerase. Many attempts have been made to rationalize the structure–activity relationship involved in this inhibition.[2,3]

Gallo and co-workers studied the molecular structure of some rifamycins by ^{1}H NMR and conformational analysis[4] and also by IR spectroscopy.[5] They concluded that the molecular structure in solution (organic solvents) is identical with the solid-state structure of rifamycin B[6] (RIFB).

Other authors studied the ^{1}H NMR spectra of various C-3 derivatives of rifamycins S and SV in different solvents.[7,8] They find that rifamycin SV derivatives are rather rigid whereas in rifamycin S derivatives the central part of the *ansa* chain bends as a function of the distance C(3)–X (where X is the substituent in position 3); they claim that these conformational variations might influence biological activity.

Recently[9] we studied the crystal structure of 3-(carbomethoxy)rifamycin S (RIFCMS) and found that this structure is somewhat different from those previously found for rifamycin B[6] and rifampicin[10] (RIFMPSV). We also demonstrated[9] the existence in solution for both 3-(carbomethoxy)rifamycin S and SV of various conformers (see Scheme I).

All these considerations prompted us to study the ^{1}H NMR spectra of various 3-substituted S and SV rifamycins both in organic solvents and in D_2O. The purpose was to investigate whether and how the conformation changes as a function of the solvent and the substituent and to compare the results with those obtained in the solid state. Moreover, since a structure–activity relationship was put forward based on the solid-state molecular structure alone,[6,11] we thought it desiderable to investigate the molecular conformation of these antibiotics in water solution in order to support this correlation.

Crystal Data

Several ansamycins have been studied in the solid state by X-ray diffraction. They are rifamycin B *p*-iodoanilide[6] (RIFB), rifampicin[10] (RIFMPSV), 3-(carbomethoxy)rifamycin S[9] (RIFCMS), tolypomycinone tris(*m*-bromobenzoate)[12] (BRTOL), and tolypomycinone[11] (TOL).[13]

The conformation of the *ansa* chains, as found in crystals, is described in Table I, where the torsion angles along the 17-membered carbon skeleton are reported. Molecular structures are shown in Figures 1 and 2.

From this data it is possible to sketch the following observations: the central part of the carbon skeleton (from

---

[1] Presented at the Swiss-Italian Meeting on Theoretical Inorganic and Bioinorganic Chemistry, Bressanone, Italy, Mar 2, 3, 1981.

[2] M. Brufani in "Topics in Antibiotic Chemistry", Sammes, Ellis Horwood Limited, Chichester, England, 1977.

[3] S. Lancini and W. Zanichelli in "Structure–Activity Relationship among the Semisynthetic Antibiotics", D. Perlmann, Ed., Academic Press, New York, 1977.

[4] G. G. Gallo, E. Martinelli, V. Pagani, and P. Sensi, *Tetrahedron*, **30**, 3093 (1974).

[5] P. Ferrari and G. G. Gallo, *Il Farmaco Ed. Sci.*, **30**, 676 (1975).

[6] M. Brufani, S. Cerrini, W. Fedeli, and A. Vaciago, *J. Mol. Biol.*, **87**, 409 (1974).

[7] M. L. Casey and H. W. Whitlock, *J. Am. Chem. Soc.*, *97*, 6231 (1975).

[8] M. F. Dampier, C. W. Chen, and H. W. Whitlock, *J. Am. Chem. Soc.*, **98**, 7064 (1976).

[9] M. Brufani, L. Cellai, S. Cerrini, W. Fedeli, A. Segre, and A. Vaciago, submitted for publication in *J. Chem. Soc., Perkin Trans. 2.*

[10] M. Gadret, M. Goursolle, J. M. Leger, and J. C. Colleter, *Acta Crystallogr., Sect. B*, **B31**, 1454 (1975).

[11] M. Brufani, L. Cellai, S. Cerrini, W. Fedeli, and A. Vaciago, *Mol. Pharmacol.*, **14**, 693 (1978). Tolypomycinone differs from rifamycin S in having C-18 as a carbonyl and C-31 as a methylenic bridge connecting C-19 and C-20, instead of the C(18)=C(19) double bond.

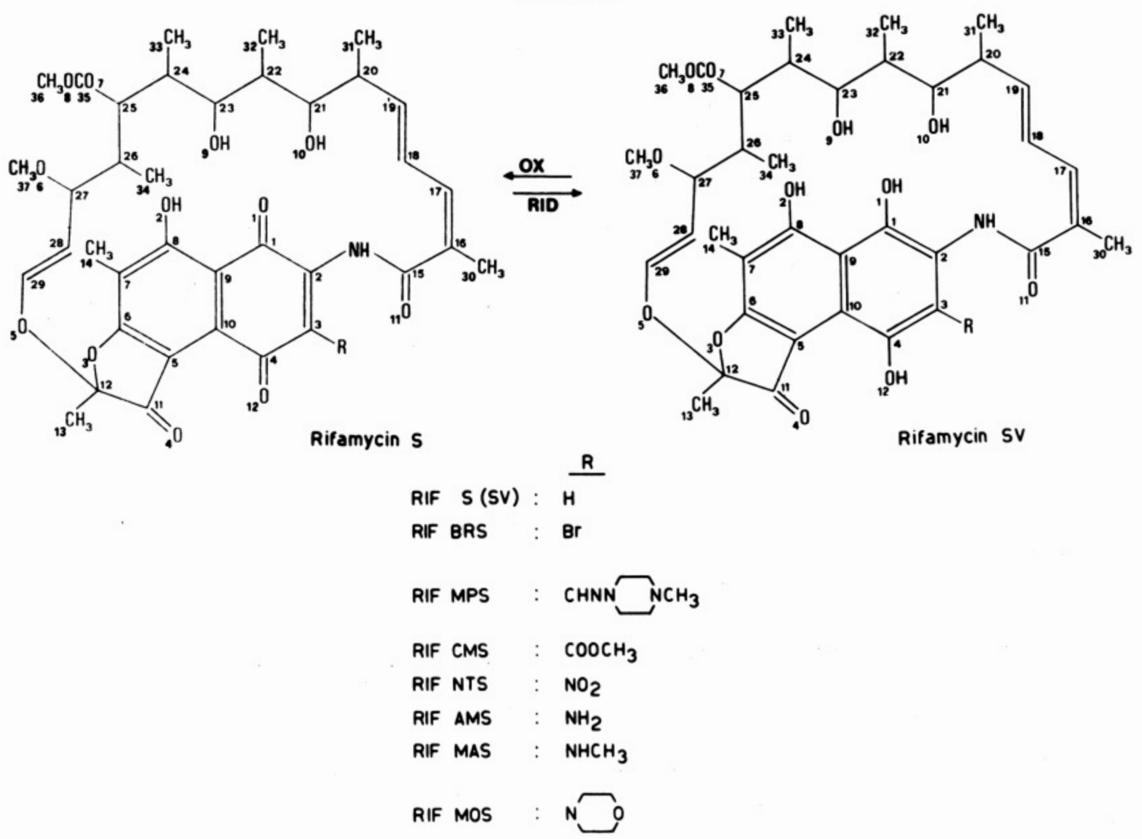
[12] K. Kamiya, T. Sugino, Y. Wada, M. Nishikawa, and T. Kishi, *Experientia*, **25**, 901 (1969).

[13] The X-ray structures of rifamycin Y *p*-iodoanilide[6] and of rifamycin S iminomethyl ether [S. K. Arora, *Acta Crystallogr., Sect. B*, **B37**, 152 (1981)] have not been considered in our discussion because the chemical modifications do not make them directly comparable with the other compounds.

[14] W. Klyne and V. Prelog, *Experientia*, **16**, 521 (1960).

0022-3263/82/1947-2652$01.25/0

Scheme I



	R
RIF S (SV) :	H
RIF BRS :	Br
RIF MPS :	CHNN NCH$_3$
RIF CMS :	COOCH$_3$
RIF NTS :	NO$_2$
RIF AMS :	NH$_2$
RIF MAS :	NHCH$_3$
RIF MOS :	N O

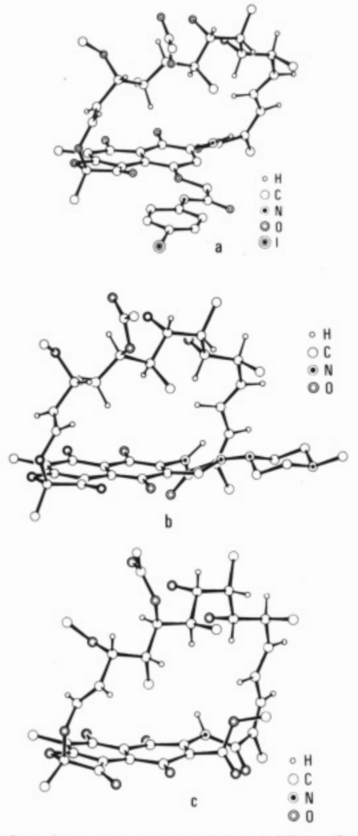


Figure 1. Molecular structures of (a) RIFB, (b) RIFMP, and (c) RIFCMS as obtained by X-rays. The same orientation of the chromophore rings for all molecules is adopted.

C-21 to C-26) always exhibits virtually the same conformation; the mean values of the five torsion angles along this part of the *ansa* chain are −173 (7)°, 59 (6)°, 176 (8)°, 167 (12)°, and 175 (14)°, respectively. For RIFB, RIFMPSV, and RIFCMS, in particular, the similarity in the *ansa* chain conformation extends from C-16 up to C-27. The following mean values (and estimated standard deviations) are found for each of the 11 angles in sequence: 4 (2)°, 164 (8)°, −173 (7)°, 20 (10)°, 173 (6)°, 178 (2)°, 57 (5)°, 175 (11)°, 161 (7)°, 169 (14)°, 174 (6)°. However, RIFCMS differs from RIFB and RIFMPSV in the torsion angles around the C(2)–N and C(15)–C(16) bonds, which define the orientation of the trans amidic group N–C(15)–O, and in the torsion angles around the bonds C(27)–C(28) and C(29)–O(5), which define the orientation of the plane of the double bond C(28)═C(29).

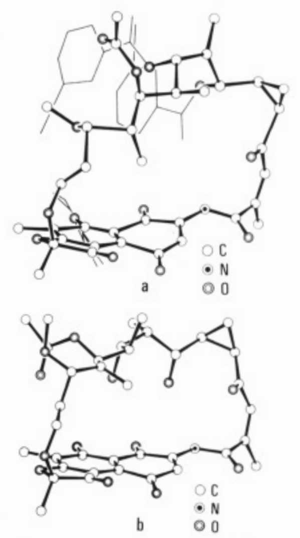


Figure 2. Molecular structure of (a) TOL and (b) BRTOL as obtained by X-rays (orientation as in Figure 1).

These changes produce only two well-localized effects in the proximity of the junctions with the chromophore rings: (i) a rotation of the amidic group as a whole around

Table I. Torsion Angles Along the Skeleton of the *ansa* Chain as Determined by X-rays[a]

atoms	angle, deg				
atoms	RIFB	RIFMP	RIFCMS	BRTOL	TOL
C(1)-C(2)-N-C(15)	−32	−55	−141	−153	−171
N-C(15)	180	179	177	175	179
C(15)-C(16)	−43	−31	63	76	80
C(16)-C(17)	5	4	2	10	−2
C(17)-C(18)	168	155	169	179	−152
C(18)-C(19)	−175	−165	−179	122	122
C(19)-C(20)	−22	−19	−30	−2	0
C(20)-C(21)	170	169	180	149	170
C(21)-C(22)	−179	−176	−178	−162	−170
C(22)-C(23)	53	62	57	53	68
C(23)-C(24)	174	165	−174	−176	173
C(24)-C(25)	155	159	169	168	−175
C(25)-C(26)	174	153	180	−169	178
C(26)-C(27)	−170	−171	180	178	67
C(27)-C(28)	117	118	−110	−138	123
C(28)-C(29)	−168	−175	−176	−161	175
C(29)-O(5)	49	65	−127	−111	42
C(29)-O(5)-C(12)-O(3)	−79	−78	−52	−56	−70

[a] The convention of Klyne and Prelog[14] has been adopted.

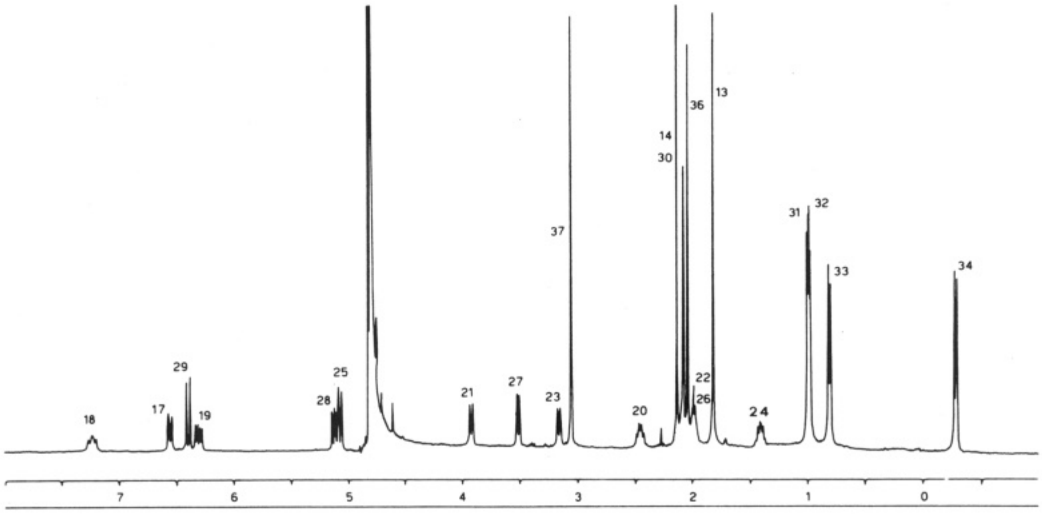


Figure 3. 400-MHz ^{1}H NMR spectrum of 3-bromorifamycin SV in D_2O.

the N–C(16) direction, so that the carbonyl at C-15 is in cisoid or transoid orientation with respect to the C(2)—C(3) bond, in the two cases; (ii) a rotation of the plane of the double bond as a whole, C(28)═C(29), which displays opposite orientations in the two cases.

These two conformational variations leave almost unaffected the position of the *ansa* chain with respect to the naphthalenic nucleus; thus the spatial relationships between the four oxygen atoms O-1, O-2, O-9, and O-10, essential for antibacterial activity,[2,3] remain very close.

Among tolypomycins, BRTOL displays a molecular shape very close to that of the rifamycins, with the amidic group and the C(28)═C(29) double bond both oriented in a way similar to that of RIFCMS; in TOL the carbonyl group is reversed as in BRTOL and RIFCMS, while the plane of the C(28)═C(29) double bond is oriented as in RIFB and RIFMPSV.[15]

These considerations suggest that in rifamycins, both in the reduced and oxidized forms and regardless of the substituent at C-3 or C-4, the central part of the *ansa* chain is rather rigid; only two regions of possibly large conformational flexibility are present, situated near the junctions with the chromophore rings. All the possibly large conformational variations occur through a rotation of 100° of

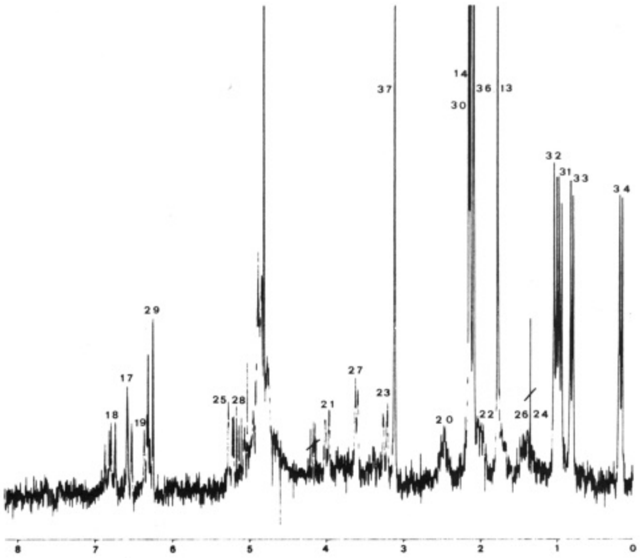


Figure 4. 200 MHz ^{1}H NMR spectrum of 3-bromorifamycin S in D_2O (water suppressed).

---

(15) Note that in TOL the two hydroxyls on C-21 and C-23 are in a very different position from that found in the other ansamycins due to a rotation of the central part of the *ansa* chain as a whole. This is accomplished on one hand through two small cooperative rotations around bonds C(17)–C(18) and C(20)–C(21) and on the other through a large variation of the torsion angle around bond C(26)–C(27).

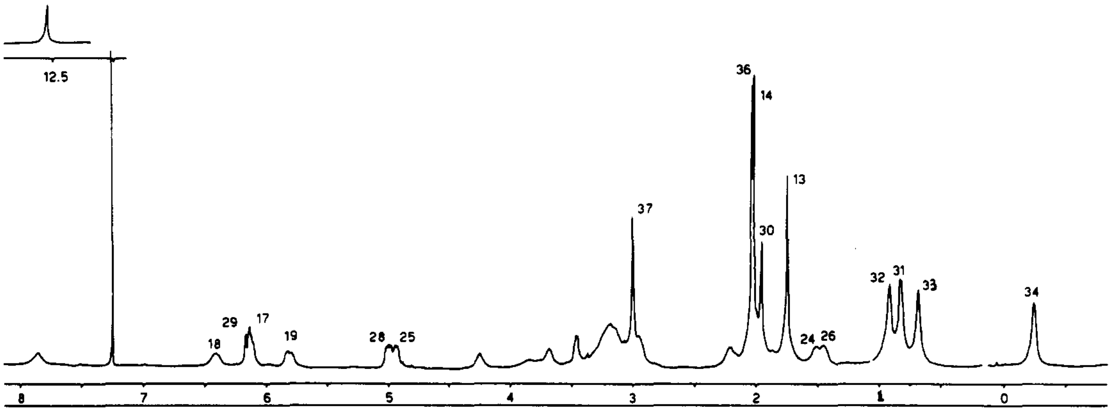


Figure 5. 400-MHz ^1H NMR spectrum of 3-bromorifamycin SV in $CDCl_3$.

Table II. Chemical Shifts[a] and Coupling Constants of Rifamycin SV Derivatives in D_2O

RIFSV	RIFBRSV	RIFMPSV	RIFCMSV	RIFNTSV	RIFMOSV		RIFSV	RIFBRSV	RIFMPSV	RIFCMSV	RIFNTSV	RIFMOSV
						HN						
						|						
						$C_{15}=O$						
						|						
						$C_{16}-CH_3$ $_{30}$	2.06	2.07	2.05	2.00	2.07	2.02
						‖						
6.36	6.55	6.52	6.45	6.47	6.51	HC_{17}						
10.5	11	11.5	11	11	11	|						
6.49	7.23	7.05	7.01	6.92	6.75	HC_{18}						
16	16	16	16	16	16	‖						
6.19	6.30	6.25	6.26	6.3∼	6.14	HC_{19}						
7.5	8	7.5	7.5	∼8	7.5	|						
2.38	2.45	2.41	2.44	2.5∼	2.42	$HC_{20}-CH_3$ $_{31}$	1.00	0.99	0.99	0.99	1.01	0.98
9.5	11	∼10	∼9	∼10	10	|						
3.83	3.92	3.84	4.05	4.2	3.82	$HC_{21}-OH$						
2	2		∼2		2.5	|						
1.92	1.98	1.9∼			1.91	$HC_{22}-CH_3$ $_{32}$	0.96	0.98	0.97	1.01	1.01	0.92
2.5	3		2		2	|						
3.13	3.15	3.14	3.17	3.19	3.14	$HC_{23}-OH$						
10.5	11	11	11	11	10.8	|						
1.36	1.40	1.4	1.47	1.6∼	1.5	$HC_{24}-CH_3$ $_{33}$	0.68_5	0.80	0.63	0.71	0.72	0.58
0	0	0	0		0	|						
5.02	5.06	5.09	5.10	5.0	5.15	$HC_{25}-OAc$ $_{36}$	2.02_0	2.04	2.07	2.06	2.0	2.10
11	11	11	11		11	|						
1.28	1.23	1.20	1.23	1.3	1.3	$HC_{26}-CH_3$ $_{34}$	-0.17_5	-0.28	-0.22	-0.23	-0.22	-0.07
1	∼1		∼1		∼1	|						
3.48	3.51	3.48	3.55	3.57	3.58	$HC_{27}-OMe$ $_{37}$	3.05_7	3.06	2.93	3.06	3.07	3.10
9.5	8.7	8.2	7.5	8.5	7	|						
5.18	5.11	5.15	5.17	5.15	5.21	HC_{28}						
13	13	13	13	13	3	‖						
6.38	6.39	6.38	6.31	6.32	6.20	HC_{29}						
						|						
						O						
						$\underset{13}{CH_3}$	1.81	1.81	1.80	1.81	1.80	1.81
						$\underset{14}{CH_3}$	2.10	2.13	2.12	2.11	2.12	2.27
						$J_{H_{27}-H_{29}}=0$						

[a] In parts per million from Me_4Si at room temperature.

the N—C(15)═O trans amidic group around the N—C(16) direction and through another rotation of 180° of the C(28)═C(29) trans double bond around the C(27)—O(5) direction.

^1H NMR Data

All spectra in D_2O are very sharp and show a single set of resonance lines for each proton; this holds both for rifamycin SV derivatives (solubility 10^{-4}–10^{-5} mol dm^{-3}) as well as for rifamycin S derivatives (solubility 10^{-5}–10^{-6} mol dm^{-3}) (Figures 3 and 4). Several rifamycin SV derivatives in $CDCl_3$ or CD_2Cl_2 show broad signals at room temperature (Figure 5). At temperatures below −70 °C, these signals split into a double set of resonance lines generally having different intensity. Some rifamycin S derivatives in organic solvents ($CDCl_3$, CD_3COCD_3) show at room temperature sharp spectra in which a double set of resonances appears for each proton (Figure 6); the relative intensity of the two sets of signals is different in different organic solvents. On addition of a trace of acid, both the rifamycin S and SV derivatives, which show either a double set of resonances or broad resonance lines, give rise to a single set of sharp lines coincident with previously reported data.[4,7,8]

Taken together these observations indicate that in D_2O only one conformer exists both for the rifamycins S and SV derivatives, while in organic solvents several rifamycin S and SV derivatives have more than one conformer. It cannot be ruled out that in D_2O also two conformations exist but rapidly interconvert.

In addition to the general considerations above, Tables II–V list chemical shifts, coupling constants, and relative assignments for some rifamycin S and SV derivatives, almost always consistent with previously reported data. It can readily be observed that the variability of chemical shifts is very low for all functional groups, with the note-

Table III. Chemical Shifts[a] and Coupling Constants of Rifamycin SV Derivatives in $CDCl_3$ Relative to the Major Isomer

RIFSV	RIFBRSV	RIFMPSV		RIFNTSV			RIFSV	RIFBRSV	RIFMPSV	RIFNTSV
	7.8	12.		8.8		HN				
						$C_{15}=O$				
						$C_{16}-C_{30}H_3$	2.05	1.95	2.07	2.03
6.2	6.2∼	6.38		6.5		HC_{17}				
			11.5							
6.3	6.4∼	6.57		6.55		HC_{18}				
			15.5		12					
5.75	5.8	5.92		6.15		HC_{19}				
			5		4.5					
2.3	2.2	2.37		2.48		$HC_{20}-C_{31}H_3$	0.8	0.8	0.87	0.95
			9		10					
3.6		3.76		4.02		$HC_{21}-OH$			3.44	3.82
			1							
		1.70		1.85		$HC_{22}-C_{32}H_3$	1.0	1.0	1.01	1.05
			2.5							
3.0		3.01		3.1		$HC_{23}-OH$			3.61	3.75
			11							
	1.5	1.53		1.7		$HC_{24}-C_{33}H_3$	0.65	0.65	0.59	0.65
			1		1					
4.9	4.9	4.93		4.95		$HC_{25}-OAc_{36}$	2.1∼	2.05	2.05	2.08
			11		11					
	1.4	1.35		1.45		$HC_{26}-C_{34}H_3$	-0.1	-0.25	-0.30	-0.2
			2		1.5					
3.5		3.47		3.58		$HC_{27}-OC_{37}H_3$	3.05	3.0	3.03	3.07
			6.8		6					
5.0	5.0	5.09		5.12		HC_{28}				
			13		13					
6.15	6.2∼	6.20		6.12		HC_{29}				
						$OH_{(1)}$			13.3	
						$OH_{(4)}$	11.7	12.5	13.2	12.7
						$OH_{(8)}$			13.3	
						$C_{13}H_3$	1.80	1.80	1.79	1.80
						$C_{14}H_3$	2.1∼	2.1	2.22	2.13
									$J_{H_{27}-H_{29}}=1.5$	
							$C_{3}H=7.9$		$C_{1'}H=8.3$	

[a] In parts per million relative to Me_4Si at room temperature.

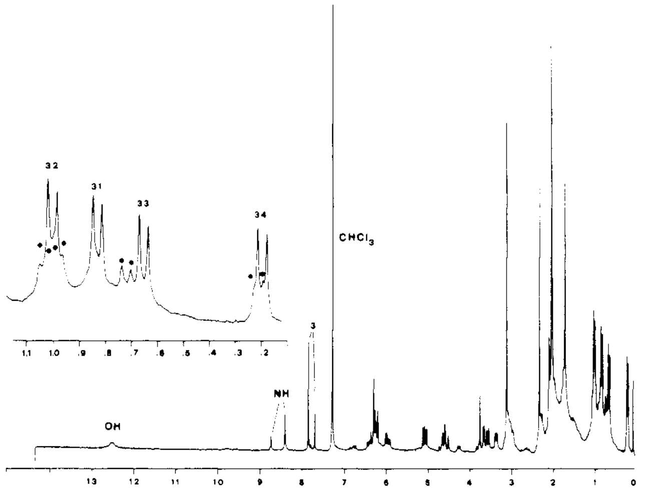


Figure 6. 200-MHz ^1H NMR spectrum of rifamycin S in $CDCl_3$.

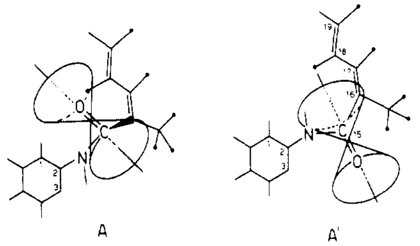


Figure 7. Sketch of the conformations of the amide plane with respect to the chromophore rings showing the anisotropic magnetic cone of the carbonyl.

worthy exception of the signal due to H-18. This proton gives rise to signals in the 6.4–6.5-ppm range or in the 6.7–7.2-ppm range, and in the case of several rifamycin S derivatives in $CDCl_3$ both signals are present. In fact, if we compare the chemical shifts of rifamycin S derivatives in $CDCl_3$, we note that they all lie within 0.2 ppm with the exception of H-25 (0.50 ppm), H-21 (0.37 ppm), and H-18 (0.83 ppm). In the case of rifamycin SV derivatives in D_2O, all shifts lie within 0.25 ppm, with the exception of H-21 (0.40 ppm) and H-18 (0.74 ppm). Finally, by comparing all the values for all the signals of rifamycin S and Rifamycin SV derivatives both in $CDCl_3$ and D_2O, we find that all shifts lie within 0.5 ppm with the exception of H-18 which has an 0.8-ppm range. Therefore, the H-18 chemical shift must be sensitive to a conformational variation inducing a strong effect on it and playing a small role on the chemical shift of all other protons. Moreover, this conformational variation cannot be related to bonds connected directly with C-18, since the coupling constants for H-(17)–H(18) and H(18)–H(19) do not change appreciably in all compounds and in all solvents. In addition, when two sets of signals are present, the H-18 multiplets show different chemical shifts but the same couplings.

The observed effects can be produced through a rotation of the plane containing the amide group around the bonds C(2)–N and C(15)–C(16) as shown in Figure 7. In fact, the rotation of the carbonyl group can induce large shifts

Table IV. Chemical Shifts[a] Coupling Constants of Rifamycin S Derivatives in D_2O

RIFS	RIF BRS	RIF MPS	RIF CMS		RIFS	RIF BRS	RIF MPS	RIFCMS
				HN				
				$C_{15}=O$				
				$C_{16}-C_{30}H_3$	2.05	2.06	2.1	2.01
6.2-6.4	6.52	6.52	6.4 ∼	HC_{17}				
	11	11						
6.2-6.4	6.78	6.73	6.5 ∼	HC_{18}				
	16	16						
5.9-6.0	6.27	6.20		HC_{19}				
	7	7						
	2.49			$HC_{20}-C_{31}H_3$	0.85	0.91	0.90	0.92
	11	10						
	3.96	3.90		$HC_{21}-OH$				
	2.5	2.5						
	2.00			$HC_{22}-C_{32}H_3$	0.95	0.99	1.0	0.98
	2.5	2.5						
	3.20	3.20		$HC_{23}-OH$				
	10.5	11						
	1.60			$HC_{24}-C_{33}H_3$	0.7	0.78	0.7	0.73
	1	1.5						
	5.22	5.22	5.12 ∼	$HC_{25}-OAc_{36}$	2.0	2.10	2.15	2.08
		11						
	140			$HC_{26}-C_{34}H_3$	0.3	0.18	0.1	0.1
	2	2						
	3.57	3.57		$HC_{27}-OC_{37}H_3$	3.15	3.10	3.10	3.10
	6.5	6.5	8					
	5.10	5.15	5.25 ∼	HC_{28}				
	13	13	13					
6.2-6.3	6.28	6.26	6.3 ∼	HC_{29}				
				O				
				$C_{14}H_3$	2.3	2.12	2.20	2.19
				$C_{13}H_3$	1.75	1.74	1.75	1.75
				$J_{H_{27}-H_{29}}=0$				

[a] In parts per million from Me_4Si at room temperature.

Table V. Chemical Shifts[a] and Coupling Constants of Rifamycin S Derivatives in $CDCl_3$

RIFS	RIFBRS	RIFCMS	RIFNTS	RIFAMS	RIFMAS	RIFMOS	
8.5	8.1	8.4	8.4	7.5	7.5	7.5	HN
							$C_{15}=O$
							$C_{16}-C_{30}H_3$
6.25	6.37	6.33	6.40	6.34	6.35	6.36	HC_{17}
10	10.5	10	10	11	11	11	
6.31	6.68	6.53	6.42	6.95	6.95	7.14	HC_{18}
16	16	16	15	16	16	16	
5.93	6.06	6.18	6.10	6.13	6.15	6.16	HC_{19}
7.5	7.5	7.2	6	6.5	6.5	6.5	
2.27	2.25	2.35	2.31	2.36	2.35	2.35	$HC_{20}-C_{31}H_3$
10	10	10	10	10.5	10	10	
3.55	3.72	3.73	3.62	3.89	3.90	3.92	$HC_{21}-OH$
0	1	1	1	∼1	0	0	
1.78	1.8	1.82	1.80	1.82	1.75	1.83	$HC_{22}-C_{32}H_3$
2	2	2	2	2	2	2	
2.98	3.04	3.01	2.98	3.03	3.05	3.03	$HC_{23}-OH$
12	10	12	10	10.8	10.8	10.8	
1.96	1.8	1.7	1.89	1.82	1.75	1.83	$HC_{24}-C_{33}H_3$
2.5		1.5	3	∼1	1	1	
4.60	5.03	4.81	4.70	5.04	5.05	5.10	$HC_{25}-OAc_{36}$
11		10.5	10	11	9.5	9.5	
1.5	1.6	1.65	1.59	1.65	1.63	1.68	$HC_{26}-C_{34}H_3$
3		2.5	3.5	3	3	3	
3.37	3.43	3.43	3.40	3.46	3.45	3.45	$HC_{27}-O-C_{37}H_3$
8		8	6	5	5	5.2	
5.06	5.03	5.07	5.02	5.09	5.01	5.07	HC_{28}
12.5	13	12.5	12.5	13	13	13	
6.20	5.96	6.13	6.15	6.06	6.05	6.05	HC_{29}
							O
							$C_{14}H_3$
							$C_{13}H_3$

	RIFS	RIFBRS	RIFCMS	RIFNTS	RIFAMS	RIFMAS	RIFMOS
$C_{16}-C_{30}H_3$	2.02	2.10	2.04	2.10	2.07	2.10	2.10
$HC_{20}-C_{31}H_3$	0.82	0.85	0.89	0.87	0.85	0.85	0.88
$HC_{21}-OH$	3.70	3.43	3.68	3.75	3.61	3.60	3.50
$HC_{22}-C_{32}H_3$	1.00	1.00	1.01	1.00	1.02	1.02	1.03
$HC_{23}-OH$	3.65	3.98	3.78	3.75	3.96	3.95	3.95
$HC_{24}-C_{33}H_3$	0.65	0.70	0.70	0.68	0.67	0.65	0.70
$HC_{25}-OAc_{36}$	2.02	2.10	2.06	2.13	2.05	2.05	2.07
$HC_{26}-C_{34}H_3$	0.20	0.20	0.20	0.25	0.09	0.12	0.18
$HC_{27}-O-C_{37}H_3$	3.10	3.10	3.10	3.12	3.08	3.10	3.09
$C_{14}H_3$	2.33	2.30	2.32	2.33	2.27	2.28	2.26
$C_{13}H_3$	1.72	1.75	1.76	1.71	1.73	1.70	1.73
$J_{H_{27}-H_{29}}$	1.5	1.5	1.5	1.5	1.5	1.5	1.5
$J_{H_{21}-OH_{21}}$	0	0	0	0	0	0	0
$J_{H_{23}-OH_{23}}$	5	∼4	5	∼4	5	5	5

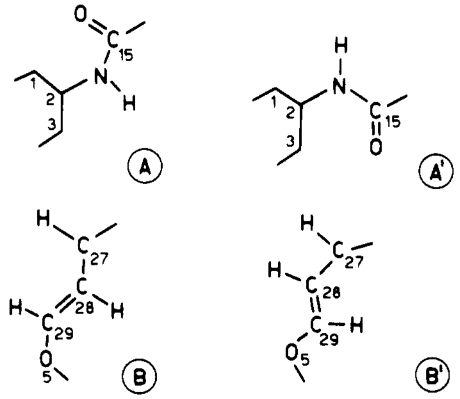
[a] In parts per million from Me_4Si at room temperature.

on H-18 (both upfield and downfield) according to the orientation of its magnetic cone, whereas it does not induce large effects on the other protons of the molecule, since all of them are quite far from the C=O dipole.

It is possible to define the appropriate conformation on considering that in the spectrum of rifampicin in $CDCl_3$ the position of the amide carbonyl is determined unambiguously. In fact an H bond clearly exists between the

Table VI. Isomers Found in Solution and in the Solid State

	AB	AB'	A'B	A'B'
Solid state	RIFB (SV) RIFMP (SV)		TOL (S)	RIFCMS (S) BRTOL (S)
D_2O solution		RIFSV (SV) **RIFCMS**(S)		RIFMP (SV) RIFBR (SV) RIFNT (SV) RIFCMSV (SV) RIFMO (SV) RIFMP (S) RIFBR (S)
$CDCl_3$ solution	RIFMP (SV) RIFNT (SV) RIFNT (S) RIFBR (S) RIFCMS (S)> RIFS (S)>		RIFMO (S) RIFMA (S) RIFCMS (S)< RIFS (S)< RIFAM (S)	



S = quinonic form SV = hydroquinonic form

> major isomer < minor isomer

amidic proton and the substituent in position 3, as evidenced by the chemical shift value of the NH proton ($\sim$12 ppm) and by the formation of a pseudoaromatic ring, δ-(H-1′) $\simeq$ 8 ppm and as also independently found by IR in the same solvent.[5]

In this case the carbonyl group will be coplanar with the chromophore and in a trans position with respect to the C(2)–C(3) bond. The contribution of the carbonyl to the chemical shift of H-18 in this case will be negligible, and this situation corresponds to a chemical shift of 6.5 ppm.[16,19]

It follows that this is the value of the chemical shift for H-18 when the carbonyl group is in a transoid conformation with respect to the C(2)–C(3) bond as shown in Figure 7A. As a consequence, the 6.7–7.2-ppm range must be associated with an "RIFCMS"-like conformation (Figure 1c), as shown in Figure 7A′, in which the carbonyl group, cisoid with respect to the C(2)–C(3) bond, exerts a downfield shift[20] on H-18.

The existence of at least two conformers in solution differing in the rotation of the amidic plane is consistent with the findings of X-ray analysis (see Table I).

Other conclusions can be drawn regarding the variations of the dihedral angle H(27)–C(27)–C(28)–H(28) by analyzing the resonances due to H-27, H-28, and H-29.

In the literature[7,8] a large variation of $J_{H27-H28}$ (in $CDCl_3$) is reported, since this coupling can have values between 4.3 and 8 Hz, a variation much larger than any variation observed for all other couplings. However, no attempt to correlate this large variation with substituent properties was successful.

We noted not only that there is a large variation in $J_{H27-H28}$ but also that an even larger effect is present. In fact, the long-range coupling $J_{H27-H29}$ is zero in all rifamycins dissolved in D_2O, whereas, this allylic coupling is always present with $J_{H27-H29}$ = 1.5 Hz in all spectra run in $CDCl_3$, CD_2Cl_2, $CDCl_3$ + DCl, or CD_3COCD_3 (see Figure 8). As a consequence, the dihedral angle H(27)–C(27)–C(28)–H(28) not only lies in the range 130–160° as previously noted[8] but also can assume values in the range

---

(16) Calculation of the screening contribution of the carbonyl on H-17, H-18, H-19, and CH_3-30 were performed by allowing the carbonyl group and the C(16)=C(17)—C(18)=C(19) fragment to rotate around the C(15)—C(16) bond, according to literature susceptibility values.[17,18] Both approximations do not differ appreciably.

(17) J. W. ApSimon and H. Beierbeck, *Can J. Chem*, **49**, 1328 (1971).

(18) R. M. Lynden-Bell and R. K. Harris in "NMR Spectroscopy", T. Nelson and Sons Ltd., London, 1971.

(19) A. L. Segre, L. Zetta, and A. Di Corato, *J. Mol. Spectrosc.* **32**, 296 (1969).

(20) W. Brugel in "Handbook of NMR Spectral Parameters", Vol. 1, Heyden & Son Ltd., London, 1979.

Table VII. H-H Dihedral Angles (Deg) for Various Rifamycins Obtained by ^1H NMR and X-ray Analysis[a,b]

	RIFCMS[d] (D_2O)	RIFMPS[e] (buffer[c])	RIFBRS[e] (buffer[c])	RIFS[d] $(CDCl_3)$	RFIBRS[d] $(CDCl_3)$	RIFCMS[d] $(CDCl_3)$	RIFNTS[d] $(CDCl_3)$	RIFAMS[e] $(CDCl_3)$	RIFMAS[e] $(CDCl_3)$	RIFMOS[e] $(CDCl_3)$
H_{17}-H_{18}		165	165	160	160	157	160	165	165	165
H_{19}-H_{20}		36	36	32	32	33	41	37	37	37
H_{20}-H_{21}	~-175	175	-175	175	175	175	175	180	175	175
H_{21}-H_{22}	~50	57	57	80	66	66	66	~70	80	80
H_{22}-H_{23}	~50	55	55	52	52	52	52	52	52	52
H_{23}-H_{24}	~-175	180	175	-175	168	-175	168	180	180	180
H_{24}-H_{25}		-62	-68	-54		-63	-50	-68	-68	-68
H_{25}-H_{26}		175		175		165	158	175	154	154
H_{26}-H_{27}		64	64	50		53	47	50	50	50
H_{27}-H_{28}	~-45	-45	-45	150	130-150	138	138	133	133	135
H_{28}-H_{29}										

	RIFNTSV[d] $(CDCl_3)$	RIFMPSV[d] $(CDCl_3)$	RIFMOSV[e] (D_2O)	RIFNTSV[e] (D_2O)	RIFCMSV[e] (D_2O)	RIFMPSV[e] (D_2O)	RIFBRSV[e] (D_2O)	RIFSV[d] (D_2O)
H_{17}-H_{18}		180	165	165	165	180	165	~160
H_{19}-H_{20}	47	45	32	30	32	32	34	32
H_{20}-H_{21}	175	164	175	175	164	175	-175	169
H_{21}-H_{22}		66	53		57		57	57
H_{22}-H_{23}		48	52		52		45	48
H_{23}-H_{24}		-175	180	-175	-175	-175	-175	175
H_{24}-H_{25}	-68	-68	-80		-80	-80	-80	-80
H_{25}-H_{26}	175	175	175		175	175	175	175
H_{26}-H_{27}	63	58	69		59		69	69
H_{27}-H_{28}	138	143	-35	-25	-33	-27	-24	-18
H_{28}-H_{29}								

	RIFBSV[d] (solid)	RIFMPSV[d] (solid)	RIFCMS[e] (solid)
H_{17}-H_{18}	165	154	169
H_{19}-H_{20}	52	45	34
H_{20}-H_{21}	174	173	-178
H_{21}-H_{22}	58	57	57
H_{22}-H_{23}	56	64	59
H_{23}-H_{24}	178	170	-175
H_{24}-H_{25}	-81	-74	-66
H_{25}-H_{26}	174	157	-178
H_{26}-H_{27}	73	64	56
H_{27}-H_{28}	177	180	-48
H_{28}-H_{29}	-169	-175	-176

[a] The 90° values reported in this table are strongly affected by errors, since near 90° the Karplus-type equations are very flat. However, we point out that the rifamycin SV derivatives in D_2O solution have $J_{H24\text{-}H25} = 0$. [b] Dihedral H-H angles in the solid state have been obtained from the geometrical positions of the hydrogen atoms calculated at 1.08 Å from the parent C_i atom, in a plane bisecting the C_{i-1}-C_i-C_{i+1} bond angle in the case of sp^2 carbon atoms, and 1.08 Å away from C_i in the direction connecting the baricenter of the three substituents and C_i for sp^3 carbon atoms. [c] Phosphate buffer (2 mM, pH 7.5). [d] Transoid for C(2)-N-C(O)-C(16). [e] Cisoid for C(2)-N-C(O)-C(16).

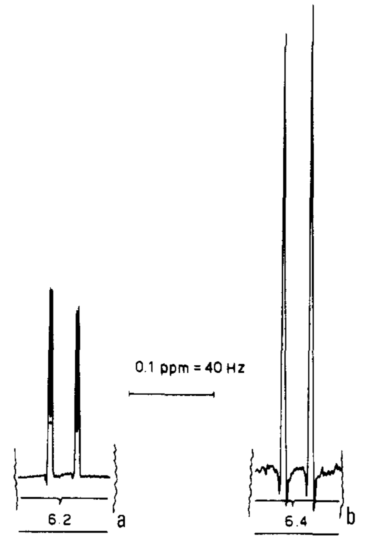


Figure 8. 400-MHz ^1H NMR spectra (resolution enhanced) of (a) rifampicin in $CDCl_3$ and of (b) 3-bromorifamycin SV in D_2O; only the portion of spectrum relative to H-29 is shown.

20–50°. The two sets of values are related neither to the oxidation state at O-1 (S or SV form) nor to the substituent in position 3 but only to the solvent.

This means that in D_2O one conformation is strongly preferred (Φ = 20–50°), while in organic solvents the other position (Φ = 130–160°) is favored. In the 20–50° conformation the hydrophobic methyl group in position 34 is slightly less exposed to the solvent, as shown in Figure 1c for the structure of RIFCMS.

Dihedral angles have been obtained by utilizing the Altona equation.[21] This equation gives rise to four solutions, which are generally not distinguishable; however, for the fragment H(27)–H(28)–H(29) in the cis–trans form (Figure 1c), the long-range coupling H(27)–H(29) must be near zero,[22] while in the trans–trans form H-27 and H-29 are generally coupled[22] (Figure 1a,b).

Thus, the choice of angles near 30° in D_2O or near 150° in $CDCl_3$ for the H(27)–C(27)–C(28)–H(28) fragment is unambiguously determined by the presence of an observable long-range coupling. Therefore, it is necessary to invoke a rotation of the plane containing the C(28)=C(29) double bond as a function of the solvent alone. This rotation is in agreement with the findings from X-ray analysis (see Table I).

Four isomers of rifamycins, three of which were found also in the solid state, can thus exist in solution. These are characterized by two average positions both of the plane H(28)–C(28)–C(29)–H(29) and of the amide plane, according to which the carbonyl group can be in a cisoid or transoid position with respect to the C(2)–C(3) bond, as illustrated in Table VI.

The *ansa* chain behaves rather like the stiff handle of a handbag having two snap fasteners. The first fastener, i.e., the orientation of the plane H(28)–C(28)–C(29)–H(29), is dominated by intermolecular interactions with the solvent. The second fastener, i.e., the orientation of the amide plane, is also dependent on the solvent; however, the influence of the nearby substituent is also observable. *In fact, all rifamycin SV derivatives studied in* D_2O *are in* the transoid form except for rifamycin SV which is in cisoid form.

Table VIII. Range of Variability of H-H Dihedral Angles

	H^1 NMR	X-rays
$C(2)-N-\overset{O}{\overset{\|}{C}}-C(16)$	cisoid transoid	cisoid transoid
$H_{19}-H_{20}$	34° ; 47°	34°; 52°
$H_{20}-H_{21}$	164° ; -175°	173° ; -178°
$H_{21}-H_{22}$	53°; 80°	57°; 58°
$H_{22}-H_{23}$	45°; 55°	56°; 64°
$H_{23}-H_{24}$	168°; 175°	170° ; -175°
$H_{24}-H_{25}$	-50° ; -80°	-66° ; -81°
$H_{25}-H_{26}$	154°; 175°	157° ; -178°
$H_{26}-H_{27}$	47°; 69°	56° ; 73°
$H_{27}-H_{28}$	-18° ; -45° 133°; 150°	-48° 177°; 180°

Some flexibility around the two snap fasteners is present, as demonstrated by the high variability of $J_{H27-H28}$ and by the spread of chemical shifts of H-18.

Let us now consider the situation of the whole *ansa* chain in solution. As mentioned before (and previously reported in the literature[4,7,8]), the variations of coupling constants are rather small and generally accompanied by small chemical shift variations. For different parts of the *ansa* chain the appropriate optimized Karplus-type equation[19,21,23] was employed to obtain dihedral angles. Since these equations give four possible solutions for each dihedral angle, *the one nearest to the findings of X-ray* structure analysis was assumed (see Table VII). This is by no means arbitrary, because all other solutions except one are incompatible with ring closure or steric hindrance,[4] and the other possible solution[4] can be ruled out due to the impossibility on the part of OH-23 to form a hydrogen bond with carbonyl 35 (as, instead, previously found by IR methods[5]).

In particular, no data were previously available on the presence of a coupling constant between OH-21 and OH-23 and their geminal proton even when chemical shifts for both OHs were reported. For both rifamycin S and SV derivatives, we measured the vicinal coupling constants of the hydroxyls in positions 21 and 23. A $\cos^2\theta$ equation[24] is applicable, with four possible solutions ($J_{180°}$ was assumed to be 6.5 Hz). Three out of these four possible solutions are in disagreement with IR data in the same solvent.[5] The fourth solution does involve those hydrogen bonds previously found by IR spectroscopy,[5] and this solution was therefore accepted. Thus a hydrogen bond between the two hydroxyls is present in all organic solutions stabilizing a rigid conformation of the central part of the *ansa* chain. This observation agrees with solid-state

---

[21] C. A. G. Haasnoot, F. A. A. M. De Leeuw, and C. Altona, *Tetrahedron*, **36**, 2783 (1980).

[22] A. A. Bothner-By in "Advances in Magnetic Resonance" Vol. 1, Waugh, J. S., Ed., Academic Press, New York, 1965.

[23] H. Booth in "Progress in NMR Spectroscopy", Vol. 5, Pergamon Press, Oxford, 1969.

[24] C. Arnaud, N. Guyen Cong Danh, and J. Huet, *Bull. Soc. Chim. Fr.*, 1603 (1972).

[25] *Most of the compounds* were kindly given by Alfa Farmaceutici SpA, Bologna, Italy. Crude samples were highly purified by column chromatography.

[26] The 200-MHz spectra have been run at the "NMR Service" of the CNR Research Area of Rome. The 270-MHz spectra have been run at Euratom, Ispra, thanks to the courtesy of Dr. H. Spiesecke. The 400-MHz spectra have been run at Bruker A. G. Karlsruhe, thanks to the courtesy of Dr. W. Hull. The technical assistance of Mr. A. Amorese, the graphic support of Mr. L. Indrizzi, and the secretarial help of Mrs. M. Colasuonno are aknowledged.

structures, in which the central part of the *ansa* chain is always in the same conformation; moreover, the same H-bond pattern was also observed in RIFCMS and TOL where the H atoms were localized.

Combining all the dihedral angles shown in Table VII, it is possible to obtain the range of variability for each angle and compare the data obtained in solution with those obtained in the solid state. The very good agreement between the two sets of data is shown in Table VIII, a possible exception being the angle H(21)–H(22) where a higher variability was found in solution.

Conclusions

The present study, in which many structures have been examined both in solution and in the solid state, leads to the conclusion that rifamycins give rise to only four kinds of isomers in spite of the apparent flexibility. These isomers are generated by the combination of two rotations, one relative to the amidic plane and the other relative to the plane containing the C(28)═C(29) double bond.

Apart from these rotations, the remaining part of the *ansa* chain between C-17 and C-27 displays only small variations. As a consequence, the spatial relationships between the four oxygens O-1, O-2, O-9, and O-10 remain the same whatever the oxidation state at O-1, the kind of C-3 substituent, or the nature of the solvent or whether the molecules are present in the solid state or in solution.

This observation is particularly important in view of the fact that the four hydroxyls (one of which, O-1, may be in the quinonic form) are responsible for the binding that leads to inhibition of bacterial DNA-dependent RNA polymerase.

Therefore, the previously proposed correlation between structure and activity derived on the basis of solid-state studies also holds in solution.

Registry No. RIFB, 13232-69-4; RIFMPSV, 13292-46-1; RIFCMS, 72393-97-6; BRTOL, 26294-93-9; TOL, 22356-23-6; RIFMPS, 13983-13-6; RIFBRS, 57375-25-4; RIFS, 13553-79-2; RIFNTS, 72788-67-1; RIFAMS, 51756-80-0; RIFMAS, 17554-98-2; RIFMOS, 19306-05-9; RIFNTSV, 72788-68-2; RIFMOSV, 16286-09-2; RIFCMSV, 81584-03-4; RIFBRSV, 59858-23-0; RIFSV, 6998-60-3.

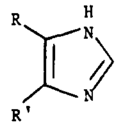
Notes

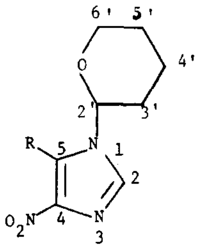
Synthesis of 4(5)-Nitroimidazole-5(4)-carboxaldehyde by Oxidative Elimination of a Nitrate Ester[1,2]

David C. J. Wu, Clair J. Cheer, Raymond P. Panzica,* and Elie Abushanab*

Departments of Chemistry and Medicinal Chemistry, University of Rhode Island, Kingston, Rhode Island 02881

Received June 8, 1981





1: R = CHO; R' = NO_2

2: R = CH_2OH; R' = H

3: R = CH_2OH; R' = NO_2

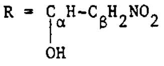
4: R = CH_2ONO_2; R' = NO_2

5: R = CO_2H; R' = NO_2

6: R = $C_\alpha H_2ONO_2$

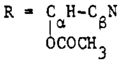
7: R = $C_\alpha HO$

8: R =



$\underset{OH}{\underset{|}{C_\alpha H}}{-}C_\beta H_2NO_2$

9: R =



$\underset{OCOCH_3}{\underset{|}{C_\alpha H}}{-}C_\beta N$

4(5)-Nitroimidazole-5(4)-carboxaldehyde (1) can serve as a precursor to a number of heterocycles of potential biological interest. It was anticipated that 1 could be prepared by controlled oxidation of 4(5)-(hydroxymethyl)-5(4)-nitroimidazole (3). An examination of the literature showed that 3 was unknown but had been proposed[3] as a possible intermediate in the oxidative nitration of 4(5)-(hydroxymethyl)imidazole (2) to the corresponding nitro carboxylic acid (5). We modified the reported procedure by lowering the reaction temperature and effected ring nitration of 2 without oxidation of the hydroxymethyl side chain. That nitration occurred was confirmed by the ^1H NMR spectrum of the crystalline reaction product which exhibited only one imidazole proton at δ 7.9. Evidence for the site of nitration as C-5(4) rather than C-2 was provided by the ^{13}C NMR spectrum of this product. The one-bond ^{13}C–^1H coupling constant for the unsubstituted carbon was 220 Hz. This value is in agreement with other published values for related C-2-unsubstituted imidazoles.[4,5] The observance of the methylene protons at δ 6.0 of this crystalline product in the ^1H NMR spectrum indicated that oxidation had not occurred; however, the large deshielding effect experienced by these protons strongly suggested that a nitrate ester had been formed and that the product was (5(4)-nitroimidazol-4(5)-yl)methyl nitrate (4). This was confirmed by elemental analysis of 4 and subsequent hydrolysis to the corresponding alcohol 3 whose ^1H NMR spectrum exhibited the expected upfield shift of the methylene protons to δ 5.0.

The literature on nitrate esters revealed that, in addition to base hydrolysis to the parent alcohol, an $E_{CO}2$ reaction[6,7] can occur to furnish the aldehyde. The aldehyde is gen-

---

[1] Presented in part at the 8th International Congress of Heterocyclic Chemistry, Graz, Austria, Aug 1981; p 61.

[2] (a) Taken in part from the Ph.D. Dissertation of D.C.J.W. University of Rhode Island. (b) Recipient of a University of Rhode Island Graduate Fellowship, 1981–1982.

[3] Kulev, L. P.; Gireva, R. N. *J. Appl. Chem. USSR (Engl. Transl.)* **1957**, *30*, 858.

[4] Dickens, J. P.; Dyer, R. L.; Hamill, B. J.; Harrow, T. A.; Bible, R. H., Jr.; Finnegan, P. M.; Hendrick, K.; Owston, P. G. *J. Org. Chem.* **1981**, *46*, 1781.

[5] Thorpe, M. C.; Coburn, W. C., Jr. *J. Magn. Reson.* **1973**, *12*, 225.

[6] Baker, J. W.; Heggs, T. G. *J. Chem. Soc.* **1955**, *616*.

[7] Buncel, E.; Bourns, A. N. *Can. J. Chem.* **1960**, *38*, 2457.

0022-3263/82/1947-2661$01.25/0