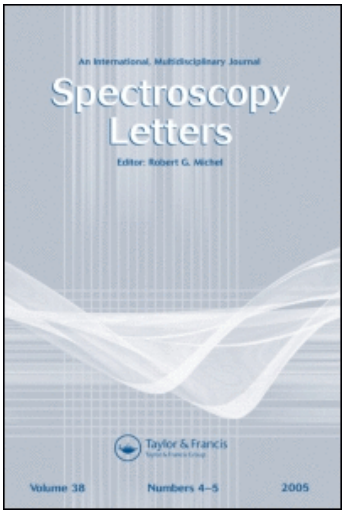

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

SPECTROSCOPIC INVESTIGATION OF THE CONFORMATIONAL PROPERTIES AND SELF-ASSOCIATION BEHAVIOR OF NATURAL COMPOUNDS IN SOLUTION

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Online publication date: 14 August 2002

To cite this Article Martini, Silvia , Magnani, Agnese , Corti, Piero , Corbini, Gianfranco , Lampariello, Raffaella , Picchi, Maria Pia , Ricci, Maso and Bonechi, Claudia(2002) 'SPECTROSCOPIC INVESTIGATION OF THE CONFORMATIONAL PROPERTIES AND SELF-ASSOCIATION BEHAVIOR OF NATURAL COMPOUNDS IN SOLUTION', Spectroscopy Letters, 35: 4, 581 — 602
To link to this Article: DOI: 10.1081/SL-120013892
URL: http://dx.doi.org/10.1081/SL-120013892

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SPECTROSCOPY LETTERS, 35(4), 581–602 (2002)

SPECTROSCOPIC INVESTIGATION OF THE CONFORMATIONAL PROPERTIES AND SELF-ASSOCIATION BEHAVIOR OF NATURAL COMPOUNDS IN SOLUTION

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ABSTRACT

The conformational properties and self-association behaviour of rifaximin and rifaximin OR (Open Ring) were investigated in solution by NMR and IR spectroscopy. The dependence of proton chemical shift on concentration and temperature were analysed to study the self-association process. IR spectra of rifaximin and rifaximin OR were also used at different concentrations to investigate the entity of specific inter- and intramolecular interactions.

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Although similar in structure the two molecules had different chemical properties in solution. This could be of some interest in view of the biological importance of this class of antibiotic molecules.

Key Words: Rifaximin; Self-association processes; Stacking interaction; Conformational analysis; Dynamical analysis

INTRODUCTION

Conformational studies are an important aspect of research into the biological functions and pharmacological properties of bioactive molecules in solution. These studies may enable better definition of structure-activity correlations.[1–3]

Function and biological behaviour are correlated with molecular structure in solution. The study of chemical properties, such as self-association processes and inter- and intramolecular interactions is important for understanding biological properties.

Rifaximin, obtained from rifamycin SV[4,5] by chemical modification of the 'naphtho-furanone' moiety, exerts antibiotic effects like rifamycin SV by inhibiting DNA-dependent RNA polymerase of bacteria[6–8] and reverse transcriptase of oncogenic and HIV RNA-viruses.[9,10] Selective electrolytic reduction of the aliphatic chain[11] of rifaximin produces an open-ring ansamycin derivative, rifaximin OR.

Combination use of different spectroscopic techniques is useful for investigating the structural properties of biomolecules in solution. The aim of this paper is to perform a qualitative and quantitative study of the stacking self-association process by analysis of the dependence of proton chemical shifts on concentration. This approach enables the self-association constants of the dimeric species of both rifaximin and rifaximin OR to be determined.

The presence of intra- and intermolecular interactions, such as ''H-bonding'' in solution were investigated by NMR and IR spectroscopy. The effects of these interactions on molecular motion were analyzed from proton and carbon relaxation rate measurements.

EXPERIMENTAL

Rifaximin (Fig. 1), 4-deoxy-4′-methylpyrido-1′,2′-1,2-imidazo-5,4c rifamycin SV was purchased from Alfa Wassermann S.p.A. Bologna, Italy

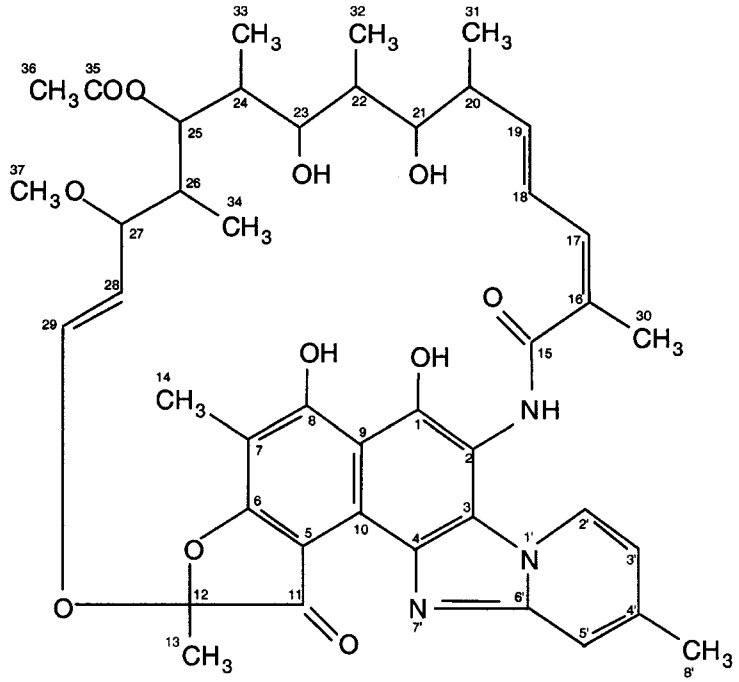


Figure 1. Structure and numbering of rifaximin.

and used without any further purification. Rifaximin OR Open Ring (Fig. 2) was obtained by electrolytic reduction of rifaximin.[11] NMR spectra were recorded on a Bruker AC-200 operating at 200.058 and 50.03 MHz for ^{1}H and ^{13}C nuclei respectively. Proton chemical shift measurements were performed using rifaximin and rifaximin OR solutions at different concentrations ranging from 10^{-3} mol dm^{-3} to 0.2 mol dm^{-3} and at different temperatures from 253K to 303K.

Proton and carbon spin-lattice relaxation measurements were obtained using the $(\pi\text{-}\tau\text{-}\pi/2\text{-}t)_n$ pulse sequence. The relaxation rates were calculated by three-parameter exponential regression analysis of the longitudinal magnetization recovery curves. The maximum experimental error in the relaxation rates measurements was 5%. The π pulse duration was 12 μs for non-selective experiments. All samples were degassed to remove dissolved paramagnetic oxygen molecules.

FT-IR spectra were recorded on a Perkin-Elmer M 1800 spectrometer in the range 4000-750 cm^{-1}. An MCT mercury-cadmium-telluride detector

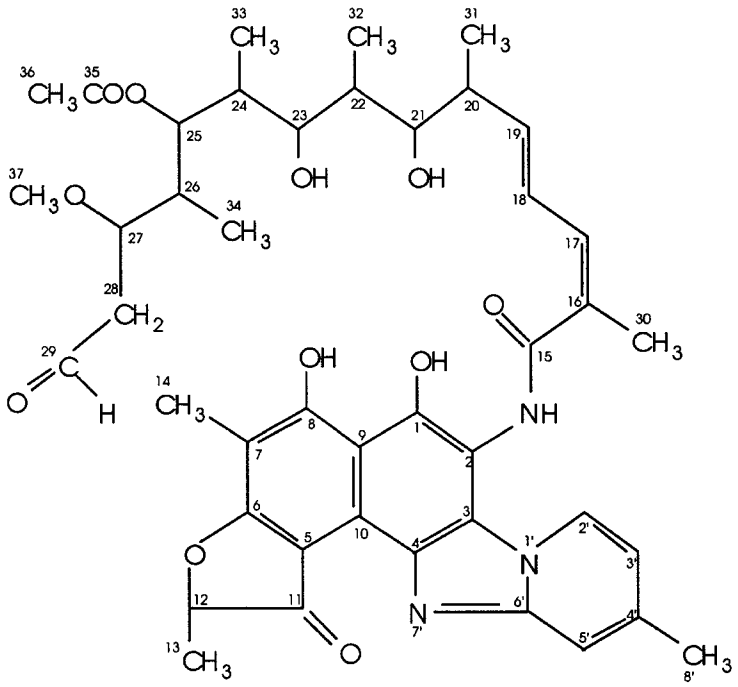


Figure 2. Structure and numbering of rifaximin OR.

was used and the apparatus was purged with nitrogen. Typically 300 scans at a resolution of 2 cm^{-1} were averaged and the spectra were stored in a magnetic system at a 1 cm^{-1} data interval. The frequency scale was calibrated internally with a reference helium-neon laser to an accuracy of 0.01 cm^{-1}. A Barnes microcircle cell for liquids, equipped with a ZnSe crystal, was used to record the spectra in $CHCl_3$ solution. The solution concentrations of rifaximin and rifaximin OR were 1.00×10^{-2} mol dm^{-3} and 1.00×10^{-3} mol dm^{-3}, respectively. The difference spectra were obtained by subtracting the spectrum of $CHCl_3$ from that of the compounds solution. The solvent subtraction was made by the null criterion, minimizing the $CHCl_3$ bands. For proton-deuterium exchange experiments, a blank of $CHCl_3$ with traces of D_2O was used. The spectra of the solid compounds were obtained with a Diffuse Reflectance apparatus by diluting the sample powders with KBr.

RESULTS AND DISCUSSION

Analysis of Inter and Intramolecular Interactions

Self Association as Detected by NMR Spectroscopy

In order to obtain information about the solution behaviour of rifaximin and rifaximin OR, the dependence of proton chemical shift on concentration was analysed. The dependence of proton chemical shift on concentration of rifaximin and rifaximin OR is reported in Figs. 3 and 4. A general decrease in proton chemical shift values was observed with increasing concentration, suggesting that rifaximin and rifaximin OR are involved in a self-association process through stacking interactions between aromatic residues of the two molecules.[12–16] This interaction causes a shielding term modulation due to a change in intensity of the anisotropic magnetic field arising from electron motion in the aromatic rings.[17–19]

To evaluate the extension of the stacking process, the analysis of proton chemical shift vs. concentration assumes a fundamental role. In rifaximin the protons most affected by the change in concentration are the aromatic protons H2′ and H5′, aliphatic protons H27 and H28, methyl protons CH_3 13 , CH_3 14 , CH_3 31, CH_3 33, CH_3 34, OH 1, OH 8 and the amide proton. The stacking interaction caused a high field shift for all protons except methyl CH_3 13 and the amide proton. In these two cases, the increase in concentration caused a low field shift, showing that formation of the dimeric species causes a decrease in anisotropic magnetic field intensity on the two protons of the rifaximin molecule.

The increasing of amide proton chemical shift values with increasing concentration suggests the presence of a high contribution due to the formation of intermolecular hydrogen bonding.[20] The different behaviour of the two OH groups indicates the presence of a high shielding contribution to the chemical shift value due to the stacking interaction. Furthermore, this suggests that the OH groups are involved in intramolecular H-bonding.

The temperature dependence of NH and OH proton chemical shift values are shown in Fig. 5. The NH proton chemical shift values show a large temperature dependence at different concentrations with typical temperature dependence values for intermolecular hydrogen-bonded protons these values are reported in Fig. 5. In particular, the NH chemical shifts show a larger temperature dependence with decreasing concentration. This confirm that the NH group of rifaximin is involved in intermolecular hydrogen bond.

On the other side, the OH groups show smaller temperature dependence, typical of intramolecularly hydrogen bonded proton.[20]

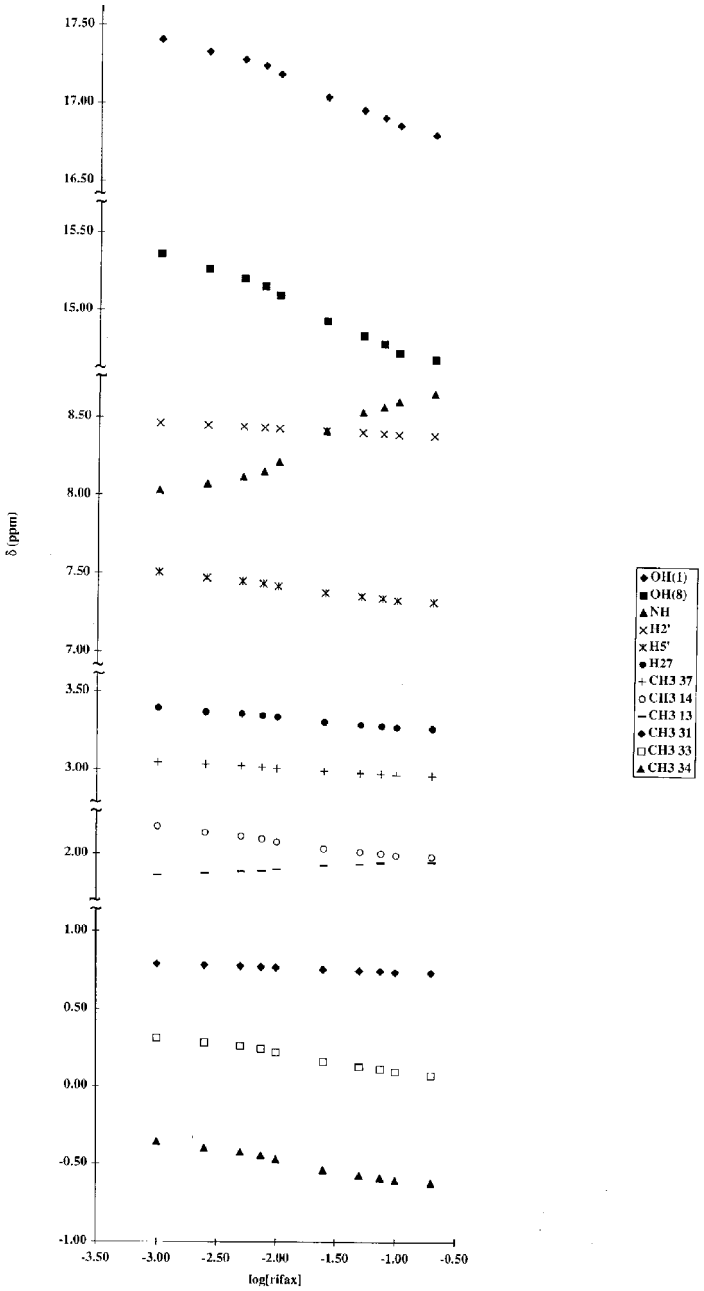


Figure 3. Proton chemical shift in relation to concentration for rifaximin (temperature = 298 K).

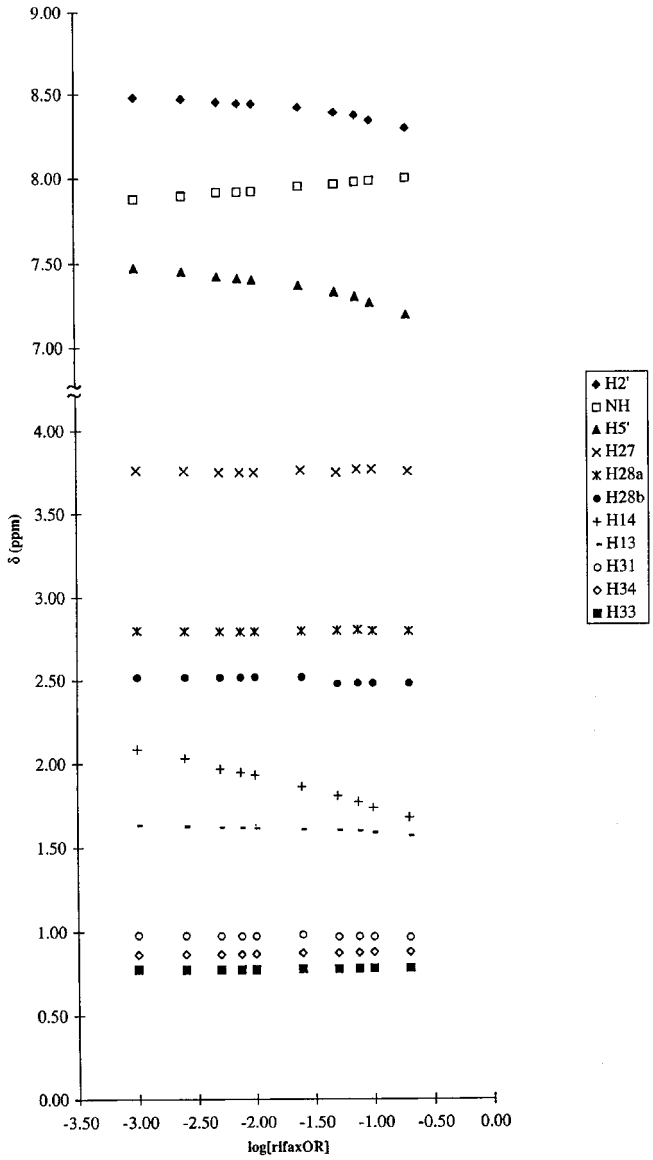
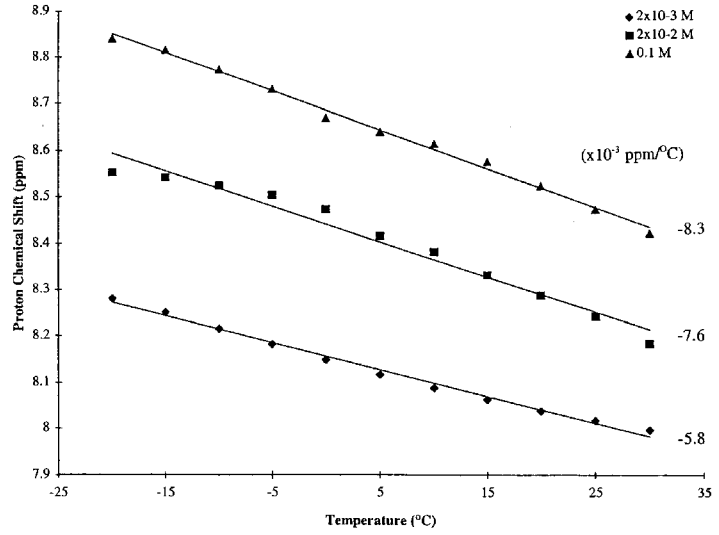
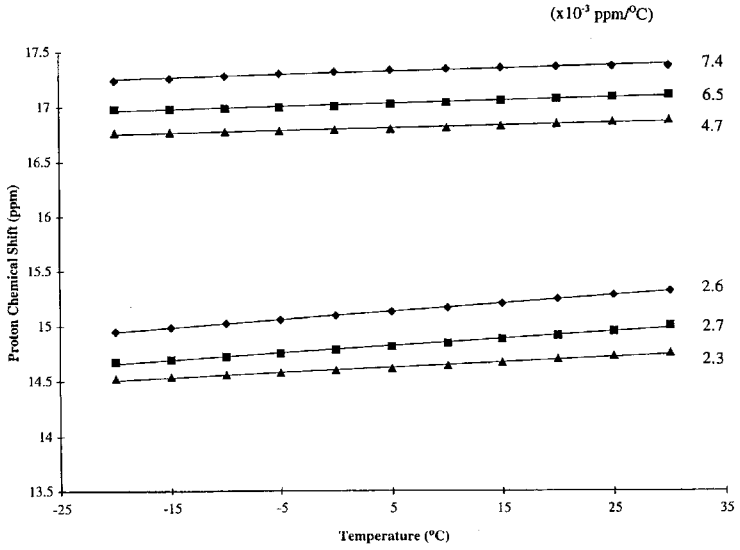


Figure 4. Proton chemical shift in relation to concentration for rifaximin OR (temperature = 298 K).



(a)



(b)

Figure 5. NH (a), OH(1) and OH(8) (b) rifaximin proton chemical shift in relation to temperature for rifaximin.

In rifaximin OR, only the aromatic protons or groups bonded to the aromatic rings showed concentration-dependent chemical shift. The aliphatic protons CH_3 34, H27, H28a and H28b were not affected by concentration changes suggesting that the break in the aliphatic chain, caused by reduction makes the aliphatic chain move away from the aromatic moiety.

The previous results were then used to calculate the association constant through stacking interaction for rifaximin and rifaximin OR. If we consider the equilibrium between the monomeric and dimeric species as follows:[21]

$$A + A \leftrightarrows A_2 \tag{1}$$

the self-association constant of the dimeric species, K_{ass}, can be derived from the following equations:

$$\Delta_{obsd} = \delta_f - \delta_{obsd} = 2(A_2/A_0)(\delta_f - \delta_c) \tag{2}$$

$$A_2 = \frac{1}{8}\left(4A_0 + \frac{1}{K} - \sqrt{8A_0\frac{1}{K} + \frac{1}{K^2}}\right) \tag{3}$$

where A_0 is the stoichiometric concentration, δ_{obsd} the observed chemical shift, δ_c the dimeric chemical shift, δ_f the free species chemical shift and A_2 the dimeric concentration.

A non-linear least-square extimation procedure implemented in the MLAB program[22] using the Marquardt-Levemberg method was used to calculate the association constants. The values for rifaximin and rifaximin OR were 63 mol^{-1} and 35 mol^{-1} respectively.

Figures 6 and 7 show the experimental and the calculated curves for self-association of rifaximin and rifaximin OR for the 2′ and 5′ aromatic protons with increasing concentration and the R^2 values of the fitting procedure.

Intra- and Inter-molecular H-Bonding and Autoassociation Phenomena: Infrared Analysis

H-bonding often 'stabilises' the molecular conformation of small and large molecules in solution or it can provide them with some peculiar characteristics, which may be important for their bioactivities.

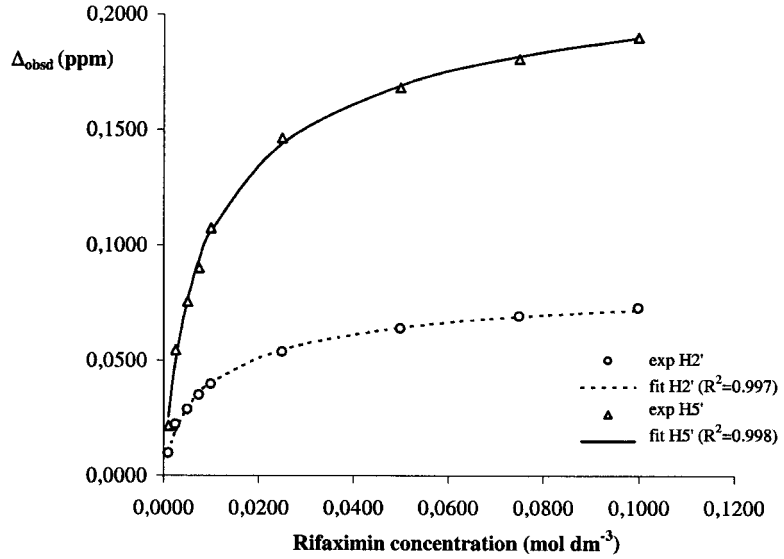


Figure 6. Experimental and calculated upfield shifts ($\Delta_{obsd} = \delta_f - \delta_{obsd}$) of 2′ and 5′ aromatic protons as a function of rifaximin concentration at 298 K.

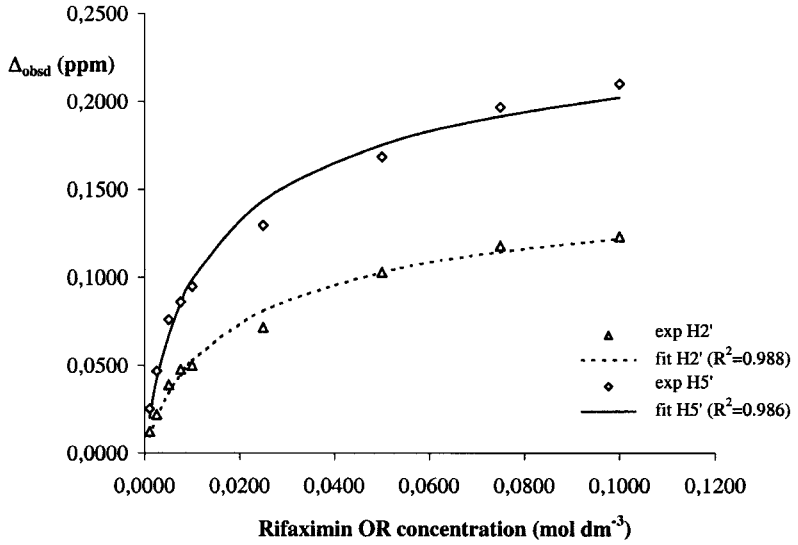


Figure 7. Experimental and calculated upfield shifts ($\Delta_{obsd} = \delta_f - \delta_{obsd}$) of 2′ and 5′ aromatic protons as a function of rifaximin OR concentration at 298 K.

The infrared spectra of solid rifaximin and rifaximin OR are shown in Fig. 8. Table 1 summarises the main wavenumbers and their assignments. The absorptions observed for rifaximin and rifaximin OR are in perfect agreement with the chemical structure of the two molecules.

The reduction process of rifaximin to rifaximin OR is revealed by the IR analysis: the IR spectrum of rifaximin OR showed aldehyde absorptions at 1702 and 2700 cm^{-1} the aldehyde C=O and aldehyde CH respectively.[23] The IR spectra of solid rifaximin and rifaximin OR revealed H-bonds involving the $COOCH_3$ moiety with the absorption wavenumbers at 1730 and 1710 cm^{-1} see Table 1. The first is due to C=O stretching of the "free COOR group", the latter to C=O stretching of the same group involved in an H-bond.[23]

The extent of inter and intramolecular H-bonding interactions is influenced by environmental factors such as temperature, nature of solvent and solution concentration.[24] The main functional groups involved in the H-bonding are the hydroxyl, carboxyl and amide moieties. Since the C=O region 1750-1600 cm^{-1} is complex to analyse, because of the superimposed absorptions of carboxyl, amide and aromatic groups, we discuss the OH and NH stretching region 3600-3000 cm^{-1}. The spectra of solid rifaximin A and its $CHCl_3$ solutions at two different concentrations [B and C] are shown in Fig. 9. The IR spectrum of the solid sample showed a series of

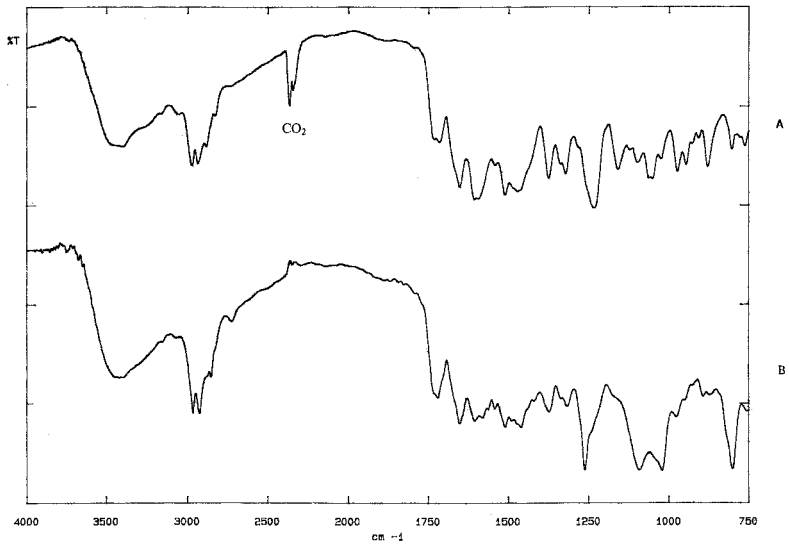


Figure 8. FT-IR spectra of solid: (A) rifaximin; (B) rifaximin OR.

Table 1. Main Wavenumbers Observed in the IR Spectra of Rifaximin and Rifaximin OR

Wavenumbers (cm^{-1})	Assignments
3450^	free OH stretching
3415	H bonded OH stretching
3270^	H bonded OH and/or NH stretching
3240*	
3005	aromatic CH stretching
2980-2800	aliphatic CH and C-CH_3 stretching
2700*	aldehydic CH stretching
1732	free amide C=O stretching of COOR
1710	H bonded C=O stretching of COOR
1702*	aldehydic C=O stretching
1670-1650	free amide C=O stretching
1650-1620	H bonded amide C=O stretching
1605-1590	aromatic C-C stretching
1550-1520	amide NH+CN stretching
1475-1430	aliphatic C-H bending
1375-1320	amide NH+CN bending
1300-750	fingerprint region

^absorption present only in rifaximin.
*absorption present only in rifaximin OR.

absorptions at 3450, 3415 and 3270 cm^{-1} suggesting that the OH groups are totally and the NH group is partially involved in H-bonding interactions.[18] The free OH stretch absorption in fact falls at 3550 cm^{-1} [23] whereas the 3450 cm^{-1} is the absorption wavenumber of the free NH.[23,24] The presence of H-bonding involving the OH and NH groups is reflected in a drop of their absorption wavenumbers.[23,24] Moreover, the wideness of their absorption band reflects a distribution of hydrogen bonds of different strength and specificity. It is well established in fact that the position and intensity of these vibrations are extremely sensitive to the strength and specificity of the formed hydrogen bonds.[25] The exact position of the absorption frequency depends very much on the strength of the H-bonding formed[26] and this bond strength strongly depends in turn on the geometry[27] such as linearity of the bands involved and the distance between the groups. Thus, the absorption at 3450 cm^{-1} is due to the stretching of OH

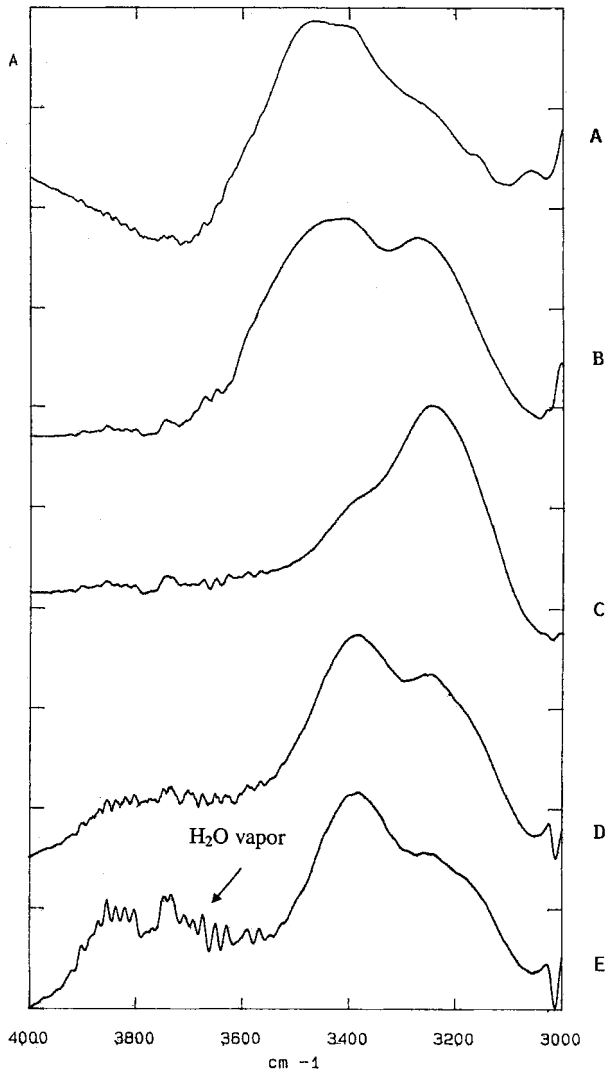


Figure 9. IR spectral region of OH and NH stretching of: (A) solid rifaximin; (B) rifaximin in $CHCl_3$ solution (concentration $= 1 \times 10^{-2}$ mol dm^{-3}); (C) rifaximin in $CHCl_3$ solution (concentration $= 1 \times 10^{-3}$ mol dm^{-3}); (D) rifaximin in $CHCl_3$ solution (concentration $= 1 \times 10^{-3}$ mol dm^{-3}) after addition of D_2O; (E) rifaximin in $CHCl_3/D_2O$ solution (concentration $= 1 \times 10^{-3}$ mol dm^{-3}) after heating. The absorbance values of spectra (D) and (E) are multiplied by a factor of 10.

groups involved in H-bonds of relatively low strength, known as "intermolecular H-bonding", superimposed to the free NH stretch;[24] whereas the 3270 cm^{-1} absorption may indicate involvement of OH and/or NH groups in very strong H-bonding interactions, here known as "intramolecular H-bonding".

Comparison of the IR spectrum of rifaximin in $CHCl_3$ at a concentration of 1.00×10^{-2} mol dm^{-3} spectrum B in Fig. 9 with that of the solid sample, does not reveal any significant shift of the infrared absorptions, but different band intensities are well evident. In spectrum B, an increase in band intensity at 3270 cm^{-1} suggests an increase in the fraction of OH and/or NH groups involved in strong H-bonding interactions. This feature becomes even more evident in spectrum C $CHCl_3$ solution of rifaximin at a concentration 1.00×10^{-3} mol dm^{-3}. In this case the intensity of the absorption at 3450 cm^{-1} sharply decreases, so that the band becomes a shoulder of the band at 3270 cm^{-1}. This suggests that in dilute solution, "intramolecular" H-bonding interactions are favored with respect to intermolecular ones.

When the strength of the H-bonding interaction, responsible for the stability of the molecular structure, was determined by 'nalyzing' the effect of the proton-deuterium exchange caused by adding D_2O to the solution, the real IR spectrum of 1.00×10^{-3} M rifaximin solution showed a very low intensity in the OH/NH absorption region due to the proton-deuterium exchange, and OD/ND absorptions appeared. When we magnified the IR absorption signals spectrum D in Fig. 9, some significant changes may be observed. The bands at 3450 and 3415 cm^{-1} again increased in intensity with respect to the band centered at 3270 cm^{-1}, restoring the situation observed for the more concentrated solution. This means that some H-bonds were broken by the proton-deuterium exchange. Moreover, the decrease in intensity at 3270 cm^{-1} suggests that the average strength of these interactions also decreased.

This feature may be explained by a "chain" of intramolecular H-bonds in the dilute solution of rifaximin. After the proton-deuterium exchange, some of these bonds are broken, leading to a situation in which only isolated intra- and/or intermolecular H-bonding interactions occur. The proton-deuterium exchange was quite a rapid process. The IR spectrum of sample D shows no significant changes on heating see Fig. 9E.

The same sequence of IR spectra for rifaximin OR is shown in Fig. 10.

The IR spectrum of the solid sample spectrum A revealed two main absorptions: one at 3415 cm^{-1} and the other at 3240 cm^{-1} attributed to the stretching of OH and/or NH groups involved in H-bonding interactions. Unlike for rifaximin, there was no band at 3450 cm^{-1} which means that the H-bonding interactions in rifaximin OR are stronger than in rifaximin. In

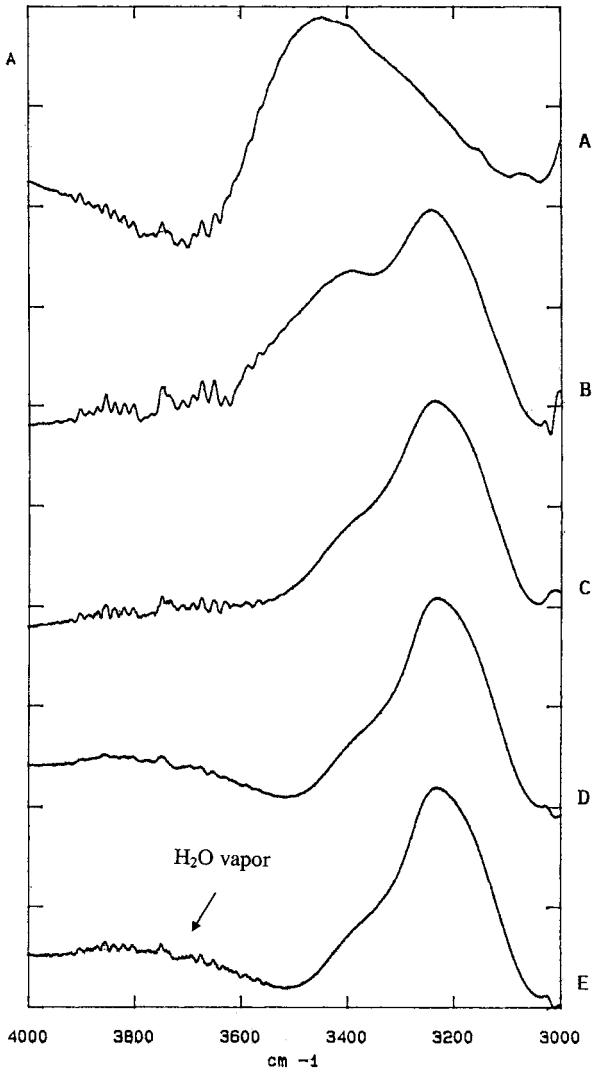


Figure 10. IR spectral region of OH and NH stretching of: (A) solid rifaximin OR; (B) rifaximin OR in $CHCl_3$ solution (concentration $= 1 \times 10^{-2}$ mol dm^{-3}); (C) rifaximin OR in $CHCl_3$ solution (concentration $= 1 \times 10^{-3}$ mol dm^{-3}); (D) rifaximin OR in $CHCl_3$ solution (concentration $= 1 \times 10^{-3}$ mol dm^{-3}) after addition of D_2O; (E) rifaximin OR in $CHCl_3/D_2O$ solution (concentration $= 1 \times 10^{-3}$ mol dm^{-3}) after heating.

this system in fact also the NH group is totally involved in H-bonding interactions. The IR spectrum of the sample in $CHCl_3$ solution at a concentration 1.0×10^{-2} mol dm^{-3} spectrum B showed a sharp increase in intensity of absorption at 3240 cm^{-1}, with respect to the band at 3415 cm^{-1}, suggesting that "intramolecular" H-bonds occur in rifaximin OR, even at the higher concentration.

When the solution was diluted to 1.0×10^{-3} mol dm^{-3} spectrum C a further increase in the band at 3240 cm^{-1} was observed, while the band at 3415 cm^{-1} became a shoulder wider distribution of strong H-bonds. Addition of D_2O spectrum D and subsequent heating spectrum E did not cause any further variation. This suggests that very strong H-bonding interactions occur in rifaximin OR. In fact, whenever several "consecutive" H-bonds were formed along this molecule the average strength of the H-bonding increased[23] and the protons strongly involved in H-bonds were not available for the proton-deuterium exchange.

ANALYSIS OF DYNAMICAL PROCESSES

The molecular motion of rifaximin and rifaximin OR were analyzed considering the inter- and intramolecular interactions and self-association processes.

The dynamical properties of rifaximin and rifaximin OR were investigated by measuring the carbon spin-lattice relaxation rate. The ^{13}C relaxation pathway of diamagnetic systems in liquid solution is dominated by intramolecular dipole-dipole interactions. Protonated carbons relax mainly by dipole-dipole interactions with a directly bound proton. The dipolar contribution to spin-lattice relaxation rates is described by the following equation:[28–31]

$$R_1^{DD} = \frac{\hbar^2 \gamma_H^2 \gamma_C^2}{10 r^6} \left[\frac{3\tau_c}{1 + \omega_C^2 \tau_c^2} + \frac{6\tau_c}{1 + (\omega_H + \omega_C)^2 \tau_c^2} + \frac{\tau_c}{1 + (\omega_H - \omega_C)^2 \tau_c^2} \right] \tag{4}$$

where $\hbar$ is Planck's constant divided by 2π, γ_H, and γ_C, are the proton and carbon magnetogyric ratios, ω_H and ω_C the proton and carbon Larmor frequencies respectively, r is the proton-carbon distance assumed to be equal to 1.08 Å and τ_c the correlation time modulating the ^1H-^{13}C interaction.

The dipolar contribution to the observed carbon spin-lattice relaxation rate, R_{1C}, can be determined by:

$$R_1^{DD} = R_1 \chi^{DD} \tag{5}$$

where R_1 is the experimental relaxation rate and χ^{DD}, the fractional dipolar term, can be calculated by comparing the experimental broad-band nuclear Overhauser effect, NOE_{BB}, with the theoretical value obtained for carbon totally relaxed by a dipolar mechanism. The fractional dipolar term measured for protonated carbons of rifaximin and rifaximin OR was found to be equal to one. The correlation time τ_c for each protonated carbon nucleus can be calculated, from the carbon spin-lattice relaxation rates and Eq. (4), assuming a constant known internuclear C-H distance. The carbon spin-lattice relaxation rates and the correlation times for all protonated carbons of rifaximin and rifaximin OR are reported in Tables 2 and 3. These values show that the aromatic and diene regions of the two molecules experience

Table 2. Experimental ^{13}C Relaxation Rates and Correlation Time Values for Rifaximin (Concentration = 0.2 mol dm^{-3}, Temperature = 298K)

Cn.	δ (ppm)	R_1 (s^{-1})	τ_c (s)
2′	129.1	7.8	4.4×10^{-10}
3′	118.6	7.5	4.1×10^{-10}
5′	110.7	7.5	4.2×10^{-10}
8′	22.7	1.4	2.0×10^{-11}
13	21.1	1.3	5.7×10^{-11}
14	7.4	1.3	1.8×10^{-11}
17	137.5	8.5	5.1×10^{-10}
18	126.1	7.7	4.3×10^{-10}
19	142.3	7.6	4.2×10^{-10}
20	38.2	5.9	3.0×10^{-10}
21	73.0	7.4	4.0×10^{-10}
22	33.2	6.4	3.3×10^{-10}
23	77.3	6.0	3.0×10^{-10}
24	37.3	6.3	3.3×10^{-10}
25	74.4	6.6	3.4×10^{-10}
26	39.0	6.3	3.2×10^{-10}
27	78.1	6.6	3.4×10^{-10}
28	116.4	6.8	1.6×10^{-10}
29	142.4	5.7	2.8×10^{-10}
30	2.9	1.3	1.8×10^{-11}
31	17.8	4.2	6.3×10^{-11}
32	11.0	2.2	3.3×10^{-11}
33	8.3	1.7	2.5×10^{-11}
34	7.8	2.4	3.6×10^{-11}
36	21.6	1.2	1.7×10^{-11}
37	57.5	1.3	1.9×10^{-11}

Table 3. Experimental ^{13}C Relaxation Rates and Correlation Time Values for Rifaximin OR (Concentration = 0.2 mol dm^{-3}, Temperature = 298K)

Cn.	δ (ppm)	R_1 (s^{-1})	τ_c (s)
2′	128.6	8.7	5.3×10^{-10}
3′	118.0	9.5	6.4×10^{-10}
5′	110.5	9.8	6.7×10^{-10}
8′	22.6	1.7	2.5×10^{-11}
12	83.6	8.2	4.8×10^{-10}
13	17.6	4.1	6.2×10^{-11}
14	7.32	1.6	2.4×10^{-11}
17	138.2	8.4	5.0×10^{-10}
18	127.8	9.6	6.5×10^{-10}
19	144.3	9.8	6.7×10^{-10}
20	41.2	8.5	5.1×10^{-10}
21	74.8	6.3	3.2×10^{-10}
22	34.1	6.1	3.1×10^{-10}
23	77.9	6.7	3.4×10^{-10}
24	37.9	7.8	4.4×10^{-10}
25	74.9	6.3	3.2×10^{-10}
26	39.0	5.1	2.5×10^{-10}
27	76.0	4.7	2.3×10^{-10}
28	46.9	3.4	7.5×10^{-11}
29	201.5	0.9	4.1×10^{-11}
30	21.7	1.8	2.7×10^{-11}
31	17.0	3.7	5.4×10^{-11}
32	12.0	2.1	3.1×10^{-11}
33	9.7	1.6	2.4×10^{-11}
34	10.8	1.7	2.5×10^{-11}
36	21.5	1.2	1.7×10^{-11}
37	58.5	1.0	1.5×10^{-11}

slower reorientation motion than the aliphatic region. The aliphatic region of the rifaximin molecule shows similar value of correlation time τ_c, indicating a stable conformation of the molecule in solution in spite of weak local dynamics. The aliphatic carbons, of rifaximin OR from carbon 15 to carbon 25 show correlation times similar to those of the aromatic carbons. Near the terminal aldehyde group, τ_c values decrease, indicating the high mobility of the terminal side of the aliphatic chain. This is in perfect agreement with IR analysis which revealed intramolecular H-bonds, also involving the carbonylic group and the amide proton of rifaximin OR. These intramolecular hydrogen bonds are responsible for stabilizing the aliphatic structure of rifaximin OR and for the uniform distribution of motion up to carbon 25.

Table 4. Experimental Non-selective Proton Relaxation Rates for Rifaximin Measured at Various Concentrations (Temperature = 298K)

	H2′	H5′	H3′	H18	H19	H25	H27	H8′	H30	H14	H32	H31	H33	H34	H37
R_1^{NS} (s−1) 0.2M	2.2	1.5	2.2	3.2	2.4	3.3	2.7	2.5	2.7	1.9	3.5	5.1	3.8	3.6	2.0
R_1^{NS} (s−1) 0.1 M	1.8	1.5	2.0	3.1	2.3	3.1	2.6	2.5	2.8	2.0	3.6	5.1	3.9	3.8	2.1
R_1^{NS} (s−1) 0.08M	1.7	1.5	1.9	3.0	2.2	2.9	2.5	2.6	2.9	2.1	3.6	5.1	4.0	3.8	2.1
R_1^{NS} (s−1) 0.05M	1.6	1.4	1.9	2.5	1.9	2.6	2.4	2.6	2.7	2.0	3.4	5.1	4.1	3.9	2.3
R_1^{NS} (s−1) 0.04M	1.6	1.4	1.8	2.3	1.9	2.2	2.3	2.5	2.9	1.8	3.3	4.9	3.9	3.8	2.3
R_1^{NS} (s−1) 0.0025M	1.3	1.1	1.8	2.3	1.9	2.2	2.0	2.4	2.9	2.0	3.4	4.9	3.9	3.9	2.1

Non-selective proton spin-lattice relaxation rates of the aromatic, aliphatic and methyl groups of rifaximin are reported in Table 4. It can be seen that the R_1^{NS} of aliphatic and aromatic but not methyl groups protons considerably depends on concentration. Methyl proton relaxation rates were lower for protons 37 and 14 suggesting that they have fast reorientational

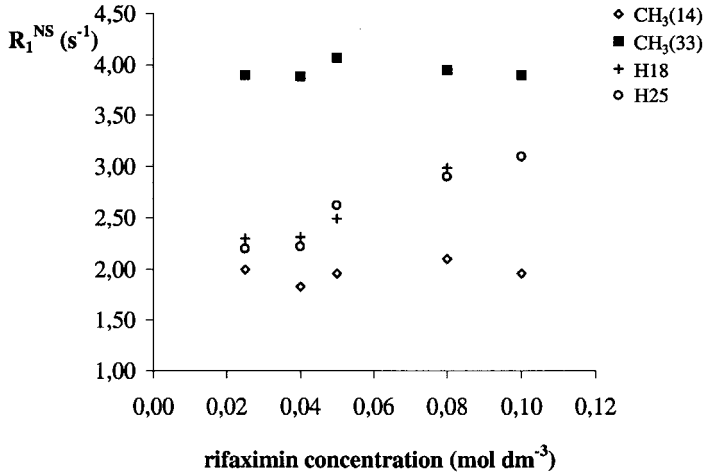


Figure 11. Non-selective proton spin-lattice relaxation rates vs. concentration of rifaximin for some aliphatic and methyl protons.

Table 5. Correlation Times Determined for a 0.2 M $CDCl_3$ Rifaximin Solution

Cn	τ_c[a] (s)	τ_r[b] (s)	τ_i[c] (s)
30	1.8×10^{-11}	3.3×10^{-10}	1.9×10^{-11}
31	6.3×10^{-11}	3.3×10^{-10}	7.3×10^{-11}
32	3.3×10^{-11}	3.3×10^{-10}	3.6×10^{-11}
33	2.5×10^{-11}	3.3×10^{-10}	2.7×10^{-11}
34	3.6×10^{-11}	3.3×10^{-10}	4.0×10^{-11}
8′	2.0×10^{-11}	3.3×10^{-10}	2.1×10^{-11}
14	1.8×10^{-11}	3.3×10^{-10}	1.9×10^{-11}
37	1.9×10^{-11}	3.3×10^{-10}	2.0×10^{-11}

[a]Effective correlation times.
[b]Overall rotational correlation times.
[c]Correlation times for internal rotation.

motion. Methyl groups 31, 32, 33 and 34 have higher relaxation rates and therefore slower reorientational motion due to steric hindrance from neighboring molecular regions. This is confirmed by the finding that the methyl relaxation rate was independent on concentration. Fig. 11 is a plot of proton spin-lattice relaxation rates vs. concentration for some aliphatic and methyl protons.

If the correlation time of the backbone determined from ^{13}C R_1 measurements is assumed to be the correlation time for the overall reorientation τ_r, then the correlation time τ_i, of the internal rotation of the methyl group is given by:[32–35]

$$\tau_c^{-1} = \tau_r^{-1} + \tau_i^{-1} \tag{6}$$

The correlation times of the methylic groups are listed in Table 5. They suggest that the proton-proton dipolar interaction is modulated by internal rotational motion with respect to the overall reorientational motion of the molecule.

CONCLUSIONS

In this study combined use of NMR and IR spectroscopies enabled definition of the dynamical and structural characteristics of rifaximin and rifaximin OR in solution. NMR analysis showed that both molecules are involved in a self-association process through stacking interactions. Infrared analysis allowed us to verify the hypothesis of a different dis-

tribution of H-bonding interactions in the two molecules. In fact, although the two molecules are very similar in the solid state, their behaviour in solution may differ very much as a consequence of the different strength and distribution of the H-bonding interactions along the molecule.

ACKNOWLEDGMENTS

The authors thank the Italian Interuniversity Consortium CSGI for financial support.

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Received February 7, 2002
Accepted May 1, 2002