MOLECULAR STRUCTURE AND CONFORMATION OF RIFAMYCIN S, A POTENT INHIBITOR OF DNA-DEPENDENT RNA POLYMERASE

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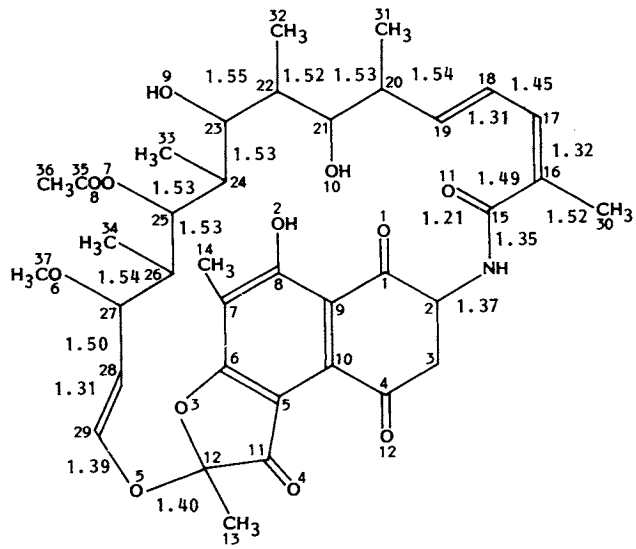
Rifamycins are antibiotics belonging to the group of naphthalenic ansamycins which exert their activity by the specific inhibition of bacterial DNA-dependent RNA polymerase (DDRP)[1]. In the presence of active rifamycins, the DDRP only synthesizes abortive RNA products[2]. They are produced from *Streptomyces mediterranei*[3] and are active against a large variety of organisms, such as bacteria, eukaroytes, and viruses, and for that reason they are some times called "wonder drugs". They are particularly useful for the treatment of tuberculosis. They have also shown antitumor activity when injected during the growth of experimental tumors[4] and also inhibit hepatitis A virus[5]. Rifamycin P is active against several mycobacterium which have been isolated from AIDS patients[6].

A great number of chemical modifications to the structure of rifamycins have been made, mostly at the position 3 or 4 of the naphthoquinone and/or naphthohydroquinone chromophore. Rifampicin, a semisynthetic 3-formylhydrazone analog of rifamycins SV, is the most active. Many studies on the relationship of structural features with the activity of these antibiotics have appeared[7~12]. These studies have indicated that the activity of the antibiotics is dependent upon the presence of (a) a naphthalene ring carrying oxygen atoms at C(1) and C(8) either in the quinone form or hydroquinone form, (b) two unsubstituted hydroxyls at C(21) and C(23) of the ansa chain, and (c) a well defined spatial arrangement of oxygen atoms at C(21) and C(23).

We have previously carried out in this laboratory a number of structural studies[9~12] on rifamycins, including orthorhombic crystals of rifamycin S, aimed at understanding the relationship between conformation and biological activity and have proposed models for possible interaction with the enzyme DNA-dependent RNA polymerase. Here we present X-ray and energy minimization studies on monoclinic rifamycin S (Fig. 1) methanol solvate to further understand the conformation of this important class of antibiotics.

Rifamycin S was kindly provided by Drs. MULLERS and SCHEIBLI of Ciba-Geigy, Basel. Data quality crystals were obtained from slow evaporation of methanol solution. The crystals belong to the monoclinic space group $P2_1$ with cell dimensions of $a=10.199(2)$, $b=9.547(2)$, $c=19.315(3)$Å, $\beta=$

Fig. 1. Chemical structure of rifamycin S with bond lengths along the ansa chain.



93.41(1)°, $Z=2$, $D_{calc}=1.285\,g/cm^3$. The intensities of 3,863 unique reflections, $2\theta<55°$, were measured using MoKα radiation ($\lambda=0.71069$Å) with a $\theta-2\theta$ scan technique, a variable scan rate (2.0~29.3°), a scan range of 2.0° and a background to scan ratio of 0.8. A total of 3,363 reflections $>3\sigma I$ were considered observed. The intensities were corrected for Lorentz and polarization effects. The unit cell parameters were obtained by least-squares fittings of the setting of 20 reflections with 2θ range of 10~25°.

Attempts to solve the structure by direct methods programs (MULTAN and SHELEX86) and molecular replacement methods (MERLOT) using the other rifamycin's coordinates as a model were unsuccessful. Finally the direct methods program SIR88[13] gave a 14 atom fragment which contained the naphthoquinone ring system. The full structure was solved by using the recycling procedure of SIR88. The structure was refined isotropically to an R factor of 0.202. At this stage a difference Fourier revealed the presence of methanol as solvent. Further isotropic and anisotropic refinement with calculated hydrogen atom positions reduced the R factor to 0.070. The refinement was based on F_{obs}, the quantity minimized being $\Sigma(F_{obs}-F_{calc})^2$. The scattering factors used were those of CROMER and MANN[14].

100 ps molecular dynamics calculations on the rifamycin S were carried out using CHARMm software in the QUANTA package by the Adopted-Basis Newton Raphson method using the X-ray coordinates. Shake constraints were used for all bonds containing hydrogen atoms. The integration time step was 0.001 ps with a bath temperature of 300 K. The coordinates of all atoms were written out for analysis every 2,000 steps of a total of 100,000 steps. The final 50 structures were energy minimized.

The stereochemistry of monoclinic rifamycin S (RS2) is shown in Fig. 2. The bond lengths and angles agree very well with those found in the orthorhombic rifamycin S (RS1)[12] and rifamycin SV (RSV)[9]. The C-O bond distance in the methanol molecule has a value of 1.332 Å. The methanol molecule exhibits high thermal anisotropy. The naphthoquinone part, as expected, is planar. The least squares plane containing 17 atoms of the ansa chain and that containing 10 atoms of the chromophore make an angle of 85°. The similar angles in RS1, RSV and rifampicin (RAMP)[15] have values of 114, 75, and 98°, respectively. Thus the orientation of the ansa chain with respect to the chromophore is somewhat similar in all active rifamycins. The overall conformation of RS2 is compared with RS1 in Fig. 3. Table 1 gives the torsion angles along the ansa chain in RS2 and compares them with those in RS1, RSV, RAMP. The conformation of RS2 differs from that of RS1 at the junction of the ansa chain and the naphthoquinone at C(2). The torsion angle N–C(15)–C(16)–C(17) has a value of 93° whereas the values observed in RS1, RSV and RAMP for this angle are 133, 119 and −31°, respectively. There is difference of 40° between RS1 and RS2. This difference clearly indicates the flexibility of the ansa chain at the C(2) junction for two polymorphs of rifamycin S. The value in RS2 is closer to the value in rifampicin as compared to RS1. We have

Fig. 2. Thermal ellipsoid plot of monoclinic rifamycin S depicting the stereochemistry.

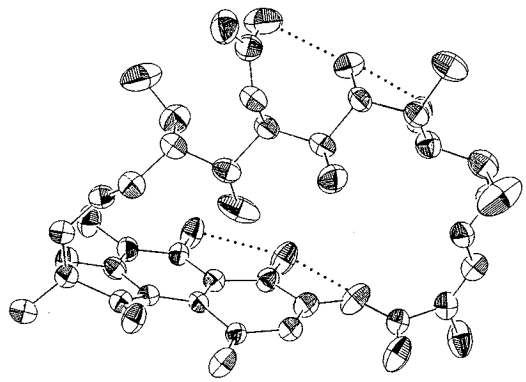


Fig. 3. Comparison of the conformation of RS1 (thick bonds) and RS2 (thin bonds).

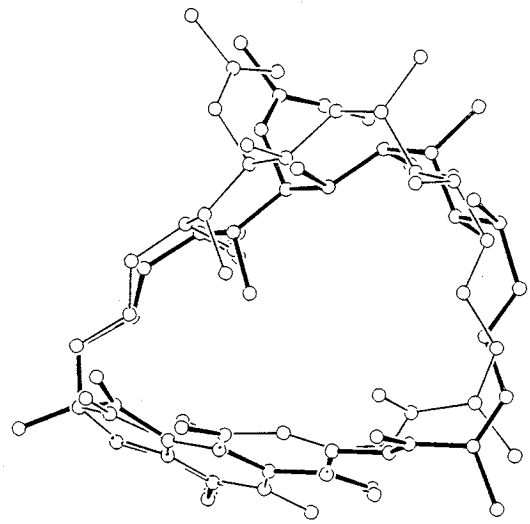


Table 1. Torsion angles (°) along the skelton of the ansa chain in the active rifamycins from X-ray, RSV (rifamycin SV), RS1 (rifamycin S, orthorhombic), RS2 (rifamycin, monoclinic), RAMP (rifampicin), and molecular dynamics energy minimized structures RS1MD and RS2MD.

Angle (°)	RSV	RS1	RS2	RAMP	RS1MD	RS2MD
C1–C2–N–C15	176	166	−171	−55	−117	−117
C2–N–C15–C16	−171	−163	−177	179	179	180
N–C15–C16–C17	119	133	93	−31	32	34
C15–C16–C17–C18	−3	−4	−3	4	20	19
C16–C17–C18–C19	−173	−174	179	155	−168	−169
C17–C18–C19–C20	−179	176	−176	−165	−171	−172
C18–C19–C20–C21	−52	−61	−46	−19	−94	−96
C19–C20–C21–C22	176	−177	176	169	−178	−178
C20–C21–C22–C23	−175	−170	−175	−176	−150	−146
C21–C22–C23–C24	62	65	61	62	79	79
C22–C23–C24–C25	180	−168	−171	165	−152	−152
C23–C24–C25–C26	176	172	173	159	166	167
C24–C25–C26–C27	174	−164	−174	153	−159	−160
C25–C26–C27–C28	−179	−165	−173	−171	−169	−169
C26–C27–C28–C29	−101	−114	−117	118	160	161
C27–C28–C29–O5	−179	−180	−178	−175	−178	−179
C28–C29–O5–C12	−118	−135	−117	65	−91	−92
C29–O5–C12–O3	−58	−65	−62	−78	−54	−54

previously noted[11,12] that the orientation at this junction is very important and is related to the activity of ansamycins. The torsion angle at the other junction C12, *i.e.* C(28)–C(29)–O(5)–C(12) has a value of −117°. This is very close to values observed in RSV and RS1 but differs significantly from those observed in rifampicin (65°). The torsion angles C(21)–C(22)–C(23)–C(24) and C(29)–O(5)–C(12)–O(3) have values of 62 and −78°. These fall within the preferred values of $59 \pm 6°$ and $-65 \pm 13°$ for other active rifamycins[12].

Fig. 4 shows the packing of the molecules in the unit cell. There is partial stacking of naphthoquinone rings. The intermolecular hydrogen bond involves the methanol proton as donor and O(10) as acceptor with a distance of 2.83Å. There are four intramolecular hydrogen bonds O(10)H–O(9), O(9)H–O(8), O(2)H–O(1) and NH–O(1) with distances of 2.89, 2.81, 2.57 and 2.59Å, respectively.

Molecular dynamics studies were carried out on RS1 and RS2, using the coordinates obtained from the X-ray studies, to see how the minimum energy structure conformation differs from the solid state. The minimization was carried out as detailed in the Methods section. The torsion angles from the energy minimized structure are given in Table 1. One observes major differences in torsion angles N–C(15)–C(16)–C(17) and C(18)–C(19)–C(20)–C(21) between the X-ray and energy minimized structure. These are also the angles which are important for the biological activity of the

Fig. 4. Packing of the molecules in the unit cell; *c* axis vertical and *a* axis horizontal.

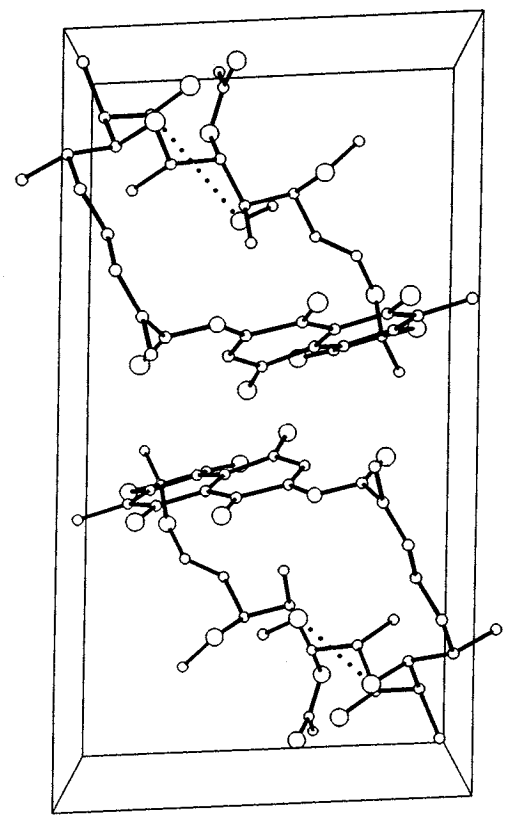
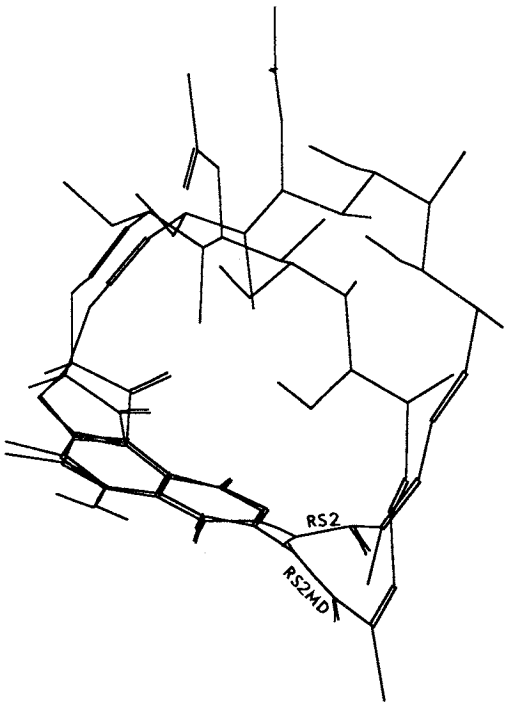


Fig. 5. Comparison of the X-ray and the energy-minimized conformation.



ansamycins. In vacuum, the torsion angle values for both RS1 and RS2 are very close, indicating that the difference in the dihedral angles in the solid state may be due to crystal packing forces. Fig. 5 compares the conformation of RS2 in the solid state and in vacuum.

This study clearly shows the flexibility of the ansa chain at the junction with the chromophore. This agrees with the NMR results[16] indicating the existence of two isomers due to the reversal of the orientation of amide and carboxyl group.

Acknowledgments

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