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 CORRELATION OF STRUCTURE  
 AND ACTIVITY IN ANSAMYCINS:  
 MOLECULAR STRUCTURE OF  
 CYCLIZED RIFAMYCIN SV

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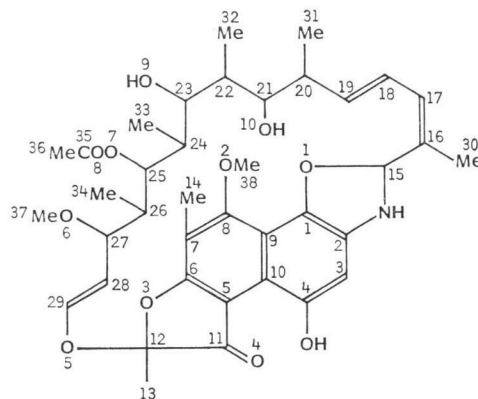
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Rifamycins are naphthalenic ansamycins having a seventeen member ansa chain. They have activity against a large variety of organisms including bacteria, eukaryotes, and viruses, and for that reason they are sometimes called "Wonder Drugs". The antimicrobial activity of these antibiotics is due to their prevention of the growth of bacterial cultures by inhibiting specifically the activity of DNA directed RNA polymerase (DDRP)<sup>1,2</sup>. These antibiotics have been used for the treatment of tuberculosis and are also active against the growth of experimental tumors<sup>3</sup>).

A great number of chemical modifications to the structure of rifamycins have been made, especially at 3 or 4 position of naphthoquinone or naphthohydroquinone chromophore. Any modification in the ansa bridge generally reduces the activity. From all the chemical and physical studies done on rifamycins, it has been concluded that the structural features necessary for the activity are: 1) presence of a naphthalene ring carrying oxygen atoms at C(1) and C(8) either in the quinone or hydroquinone form, 2) hydroxyl groups at position C(21) and C(23) of ansa chain, and 3) a well-defined spatial arrangement of oxygen atoms at C(21) and C(23).

X-Ray structural studies on both active (rifampicin<sup>4</sup>), rifamycin B<sup>5</sup>), rifamycin SV<sup>6</sup>), and 3-carbomethoxyrifamycin SV<sup>7</sup>) and inactive (tolypomycinone<sup>8</sup>), rifamycin S iminomethyl ether<sup>9</sup>) rifamycins have been carried out recently to cor-

Fig. 1. Chemical structure of cyclized rifamycin SV.

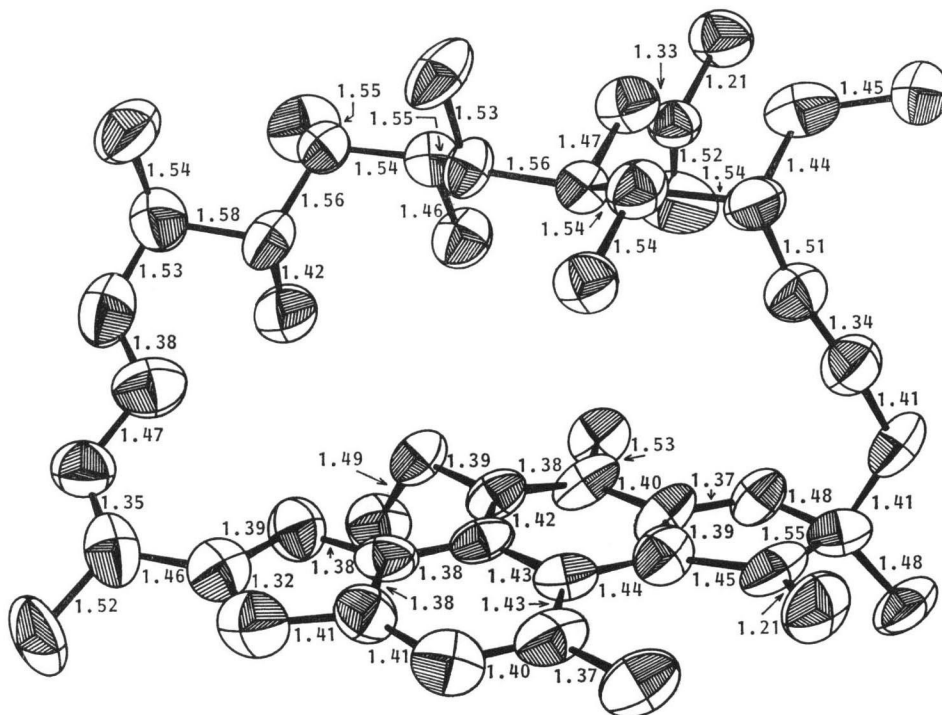


relate the structure and activity in ansamycins. LANCINI and ZANCHELLI<sup>10</sup>) have published an excellent review on structure-activity relationships in ansamycins. The present study presents the first X-ray investigation of a cyclized rifamycin (Fig. 1) and this reveals changes in the conformation of ansa chain due to cyclization.

The cyclized rifamycin SV was kindly provided by Dr. J. R. MCCARTHY of DuPont. Needle-shaped crystals were obtained from aqueous methanol. A crystal measuring 0.1 × 0.2 × 0.3 mm was used for the measurement of cell constants and data collection. The crystal data: C<sub>38</sub>H<sub>46</sub>NO<sub>11</sub>, monoclinic P2<sub>1</sub>, *a* = 20.347(7), *b* = 7.978(2), *c* = 23.364(7) Å, β = 100.6°, *Z* = 4 (two molecules/asymmetric unit), *D*<sub>c</sub> = 1.233 g/cm<sup>3</sup>. The intensities of 5720 reflections with 2θ ≥ 105 were measured using CuK<sub>α</sub> radiation (λ = 1.54178 Å on a Syntex P3/F diffractometer, using a θ-2θ scan technique, a variable scan rate (0.5 ~ 29.3 minutes), a scan range of 2.0°, and a background to scan ratio of 0.8. 3923 reflections with *I* > 3σ(*I*) were observed. The intensities were corrected for Lorentz and polarization effects.

Attempts to solve the structure (100 atoms/asymmetric unit) by direct methods program MULTAN 78<sup>11</sup>) succeeded only in obtaining partial structure. Further attempts to develop this partial structure were unsuccessful even after applying the translation function. At this stage, attempts with the latest and more powerful version of direct methods program MULTAN 82

Fig. 2. The thermal ellipsoid plot of the molecule with bond lengths (average of the two molecules).



succeeded in revealing the complete structure. The initial R factor with all the 100 nonhydrogen atoms included was 0.291. Three cycles of isotropic least-squares refinement reduced R to 0.079. Attempts to locate H atoms in the difference Fourier map were only partially successful and so hydrogen atoms were not included in the refinement. The refinement was based on  $F_o$ , the quantity minimized being  $\sum(F_o - F_c)^2$ . The weighing scheme used was based on counter statistics as defined by CORFIELD *et al.*<sup>12</sup>, the value of  $P$  being 0.04. The scattering factors used were those of HANSON *et al.*<sup>13</sup>.

The bond lengths and angles in the two molecules in the asymmetric unit agree reasonably well. The cyclization does not seem to affect the lengths of bonds that are involved in the cyclization, *e.g.* C(1)–C(2), C(2)–N, C(15)–N when compared to similar bonds in rifamycin SV.

The stereochemistry of the molecule is shown in Fig. 2. The molecule consists of a planar part formed by a five membered ring containing atoms C(1), C(2), N, C(15), O(1) attached to modified naphthoquinone ring and a fifteen membered chain. The mean deviation for plane through C(1)–C(10) is 0.2 Å. The five membered

ring involving C(1), C(2), N, C(15) and O(1) is almost planar and makes a dihedral angle of 2.0° with the least-squares plane through atoms C(1) to C(10). The other five membered ring involving C(5), C(6), C(11), C(12), O(3) makes an angle of 0.5° with plane through C(1)–C(10). The double bonds C(16)–C(17), C(18)–C(19) and C(28)–C(29) have *cis*, *trans*, and *trans* configuration respectively. The least-squares plane containing atoms C(16) through O(5) of the ansa chain and the chromophore make an angle of 46.7° in the present study, as compared with 75.1° in rifamycin SV<sup>8</sup>.

The spatial arrangement of the four atoms O(1), O(2), O(9) and O(10), which is an important factor in the binding of the rifamycins to DDRP,

Fig. 3. Spatial arrangement of O(1), O(2), O(9) and O(10) in active (left) and inactive (right) rifamycins.

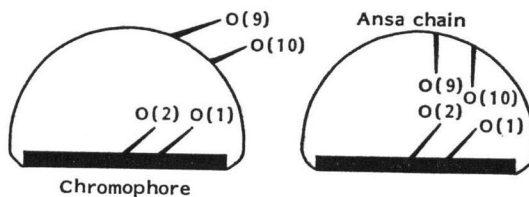
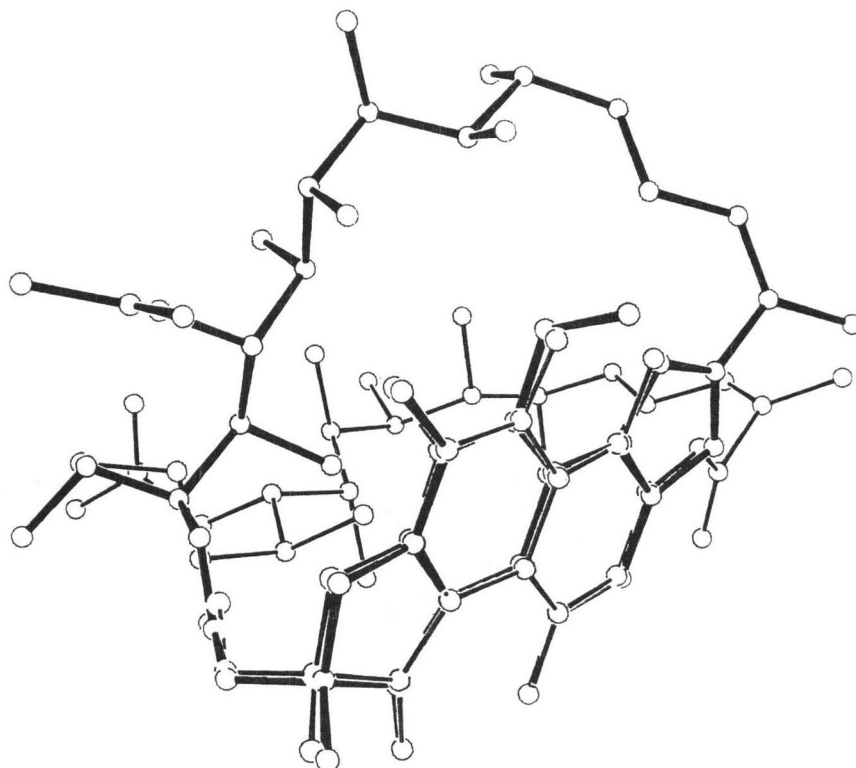


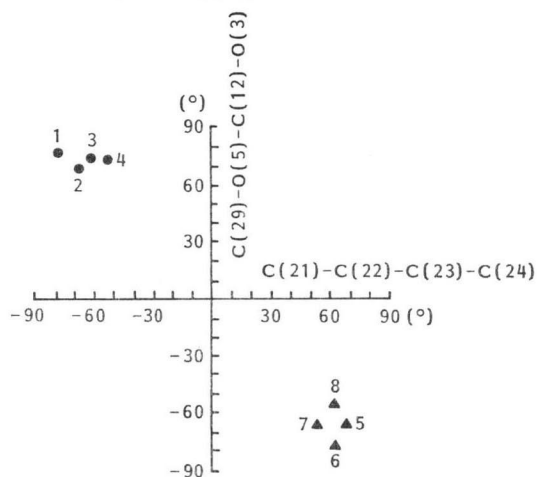
Fig. 4. Comparison of the conformations of cyclized rifamycin SV and rifamycin SV.



is different in the present study than that found in rifampicin, rifamycin SV, and rifamycin B. This is expected because of the cyclization involving O(1) and also having a methoxyl rather than a hydroxyl group at C(8). The bonds C(21)-O(10) and C(23)-O(9) are pointed toward the planar part of the molecule rather than parallel to it. This is similar to that observed in inactive rifamycins. Fig. 3 shows the spatial requirement for the active and inactive rifamycins.

Fig. 4 shows the comparison of the conformation of cyclized rifamycin SV molecule and rifamycin SV. There is vast difference in the conformation of ansa chain. Scanning through torsion angles along the ansa chain, one observes an interesting feature; *i.e.*, in all the rifamycins that have activity against DDRP, (3-carbomethoxyrifamycin, rifamycin B, rifampicin, rifamycin SV), the torsion angle C(21)-C(22)-C(23)-C(24) has values in the range of  $56 \pm 5^\circ$ , while in the case of inactive rifamycins (cyclized rifamycin SV and rifamycin S iminomethyl ether) the values are in the range of  $-60 \pm 12^\circ$ . Similar results are observed in the case of torsion angle C(29)-O(5)-

Fig. 5. Preferred values for torsion angles C(21)-C(22)-C(23)-C(24) and C(29)-O(5)-C(12)-O(3) for active [3-carbomethoxyrifamycin SV (5), rifampicin (6), rifamycin B (7), rifamycin SV (8)] and inactive [cyclized rifamycin SV (1, 2), rifamycin S iminomethyl ether (3, 4)].



C(12)-O(3) where the values are:  $-71 \pm 5^\circ$  and  $65 \pm 13^\circ$  for active and inactive rifamycins. Both

these torsion angles involve areas of ansa chain which are important for activity. One could say that these angles have preferred values. Fig. 5 shows the interrelationship between these two angles. The only exception to the above hypothesis is tolypomycinone<sup>8)</sup>.

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