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Structure-Activity Relationships in the Ansamycins: the Crystal Structure of Tolypomycinone

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SUMMARY

BRUFANI, MARIO, CELLAI, LUCIANO, CERRINI, SILVIO, FEDELI, WALTER & VACIAGO, ALESSANDRO (1978) Structure-activity relationships in the ansamycins: the crystal structure of tolypomycinone. *Mol. Pharmacol.*, 14, 693-703.

The structure of tolypomycinone, $C_{37}H_{43}NO_{13}$, obtained by mild hydrolysis from the antibiotic tolypomycin Y, was studied by X-ray methods in order to carry out further investigations on structure-activity relationships in the ansamycins. Accurate examination of the conformation of tolypomycinone led to the hypothesis that its low activity may be related to the spatial disposition of the hydroxyls on C(21) and C(23), unfavorable to the formation of hydrogen bonds with bacterial RNA polymerase. The results confirm that the activity of ansamycins is strongly influenced by the ansa chain conformation. Furthermore, we suggest that substituents introduced at position 3 in the aromatic nucleus of this class of antibiotics influence the ansa chain conformation.

INTRODUCTION

Tolypomycin Y (I), an antibiotic belonging to the ansamycins, has been isolated from a culture of *Streptomyces tolypophorus* (1). This antibiotic is very active against Gram-positive bacteria (2) and less so against Gram-negative bacteria. Its activity, like that of the rifamycins and streptovaricins, is due to the inhibition of bacterial DNA-dependent RNA polymerase (3, 4).

Tolypomycin Y is unstable and easily undergoes hydrolysis (5) to give tolyposamine (II), an amino deoxysugar, and tolypomicinone (III). Tolypomycinone is structurally related to rifamycin S (IV), but the two differ in the ketonic group at C(18), in the methylenic bridge connecting atoms C(18) and C(20), and in the absence of the C(18)—C(19) double bond. Unlike rifamycin S, tolypomycinone shows poor antibacterial activity; it inhibits the growth of *Staphylococcus aureus in vitro* only at concentrations of at least 0.5 μg/ml (6). The equivalent inhibitory concentration of rifamycin S is 0.005 μg/ml. This difference cannot be attributed to differences in ability to penetrate the bacterial wall, since the two antibiotics have very similar physicochemical properties, such as solubility and partition coefficients. Neither can it be explained why tolypomycinone is less active than tolypomycin Y, since the loss of the glycosidic moiety from the C(4) position of the naphthoquinone ring does not affect the sites in the molecule that are thought to be related to antibacterial activity.

In fact, the variations in antibacterial activity related to chemical modifications of the molecules (7) and the structural analogies among the rifamycins and tolypomycins examined suggest that the antibacterial activity of this class of antibiotics is

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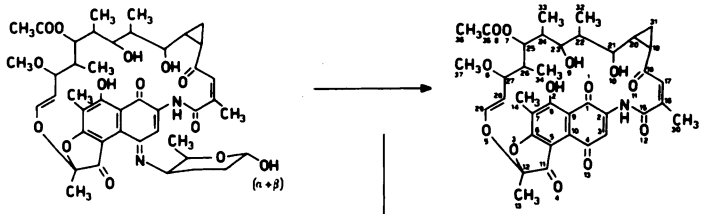
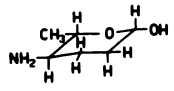
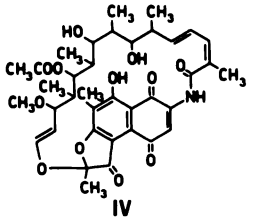


I

III



II



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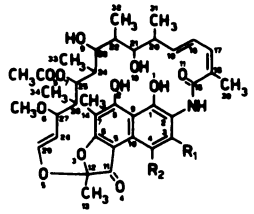


V R_1=H R_2=$-OCH_2CONH-$⟨◯⟩$-I$

VI R_1=$-CH=N-N$⟨◯⟩$N-CH_3$ R_2=$-OH$

closely connected with the presence of (a) a naphthalenic ring carrying 2 oxygen atoms [either in the quinone form or as free hydroxyl groups at the C(1) and C(8) positions] and (b) two free hydroxyl groups on C(21) and C(23), with the ansa chain in a conformation that leads to a definite geometrical relationship between the two hydroxyls and the aromatic nucleus (8).

The inhibitory activity of the rifamycins and tolypomycins is due to the formation of a complex between the antibiotic molecule and the bacterial DNA-dependent RNA polymerase. It has been shown that the formation of a complex is due to physical forces, such as hydrogen bonds and π-π interactions (9). We suggest that the activity is due to a particular conformation of the ansa chain, i.e., the conformation that makes it possible for the 2 hydroxyl oxygens on C(21) and C(23), together with the 2 oxygens on C(1) and C(8), to form intermolecular hydrogen bonds with bacterial polymerase, the first two as donors and the latter as acceptors.

The structure of tolypomycinone 8,21, 23-tri-*m*-bromobenzoate has been determined by X-ray methods and is shown in Fig. 1 (10). This derivative has no antibacterial activity, as the hydroxyl groups on C(8), C(21), and C(23) are esterified, but the ansa chain in the region around C(21) and C(23) has the same conformation as that found (8) in rifamycin B *p*-iodoanilide (V) (Fig. 2) and, more recently (11), in rifampicin (VI) (Fig. 3), both of which are very active. In all these compounds the hydroxyl groups on C(21) and C(23) have their C—O bonds in a plane that lies almost parallel to the naphthoquinone ring. Furthermore, all 4 oxygens at C(21), C(23), C(1), and C(8) protrude on the same side of an almost ideal plane containing the ansa chain atoms. This position of the hydroxyl groups seems to us to be the most favorable for antibacterial activity.

The low level of activity found in the unsubstituted tolypomycinone suggests that in this molecule the ansa chain conformation, particularly around atoms C(21) and C(23), could be altered in a manner that would make the two hydroxyl groups unavailable for intermolecular hydrogen bond formation, thus making improbable the formation of a complex with the bacterial enzyme. We have determined the molecular structure of tolypomycinone by X-ray methods in order to test this hypothesis

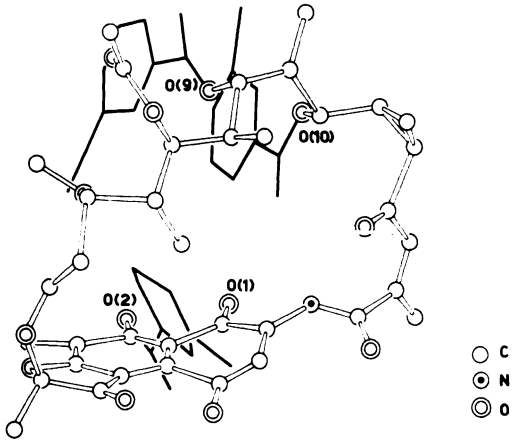


FIG. 1. *Molecular structure of tolypomycinone 8, 21, 23-tri-m-bromobenzoate (non-hydrogen atoms only)*

Coordinates are from Kamiya *et al.* (10).

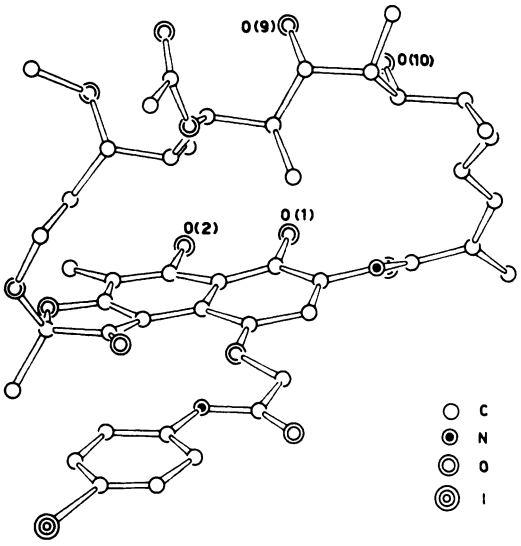


FIG. 2. *Molecular structure of rifamycin B p-iodoanilide (non-hydrogen atoms only)*

Coordinates are from Brufani *et al.* (8).

and to verify the stereochemistry that is required by the rifamycins and tolypomycins to interact with bacterial RNA polymerase. If the low activity of tolypomycinone were not due to conformational variations with respect to the active compounds, our hypothesis would have to be reconsidered.

CRYSTAL STRUCTURE DETERMINATION

Crystals of tolypomycinone were grown from a water-acetone solution. They are orthorhombic: $C_{37}H_{43}NO_{13} + H_2O$; a = 11.186 ± 0.002, b = 17.391 ± 0.003, c = 18.954 ± 0.004 A, U = 3687 A^3, D_c = 1.311 for Z = 4 and F.W. = 727.77, $F(000)$ = 1544. Space group, $P2_12_12_1$ (D_4^2, No. 19) from systematic absences; MoK$_\alpha$ radiation, λ = 0.71069 A, μ (MoK$_\alpha$) = 1.1 cm^{-1}, crystal dimension 0.2 × 0.4 × 0.4 mm. X-ray measurements were carried out on a Syntex $P2_1$ automated diffractometer, with graphite-monochromatized MoK$_\alpha$ radiation. The unit cell parameters were determined by a least-squares calculation of the 2θ values of 15 selected reflections. Intensities were collected at room temperature by ω-scan technique up to $\sin\theta/\lambda$ = 0.66. Data were recorded with a scan rate between 1° and 29° min^{-1}, depending on the intensity, and with a scan range of 0.80° centered on the calculated Bragg scattering angle. Periodic checks of the intensity values of three standard reflections did not reveal any significant X-ray damage or crystal decay. Corrections for absorption or extinction were not applied. Estimated standard deviations of the intensities were based on counting statistics. From among the 5000 measured diffraction data, 4110 unique reflections with $F_0^2 > 2\sigma$ (F_0^2) were selected as observed structure amplitudes.

The crystal structure was solved by direct methods using the MULTAN program (12), applied to 393 reflections with the highest | $E(hkl)$ | values. One of the best sets of phases was used to calculate an E map, which revealed 41 chemically signifi-

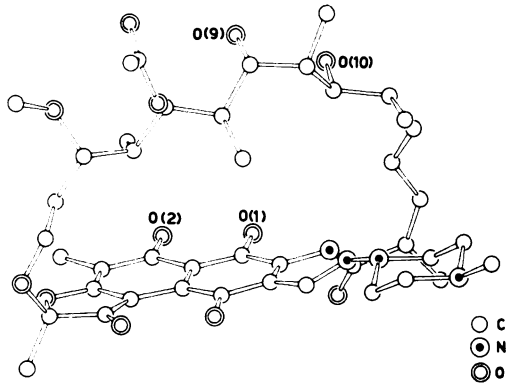


FIG. 3. *Molecular structure of rifampicin (non-hydrogen atoms only)*

Coordinates are from Gadret *et al.* (11).

cant peaks. At this stage the reliability value R was 0.43, assuming carbon form factor and thermal parameter $B = 3.0$ A^2 for the 41 peaks. The remaining part of the molecule and a water solvent molecule were recognized by usual Fourier procedures. The chemical species were assigned by electron density analysis of the peaks in the Fourier synthesis, by local analysis of the dependence of the thermal parameters on the choice of scattering form factors for each atom, and by chemical and geometrical considerations. The crystal structure was refined by block-diagonal (9×9) least-squares methods up to an R value of 0.086, with anisotropic temperature factors for all the heavy atoms. At this stage all the hydrogen atoms were found by difference Fourier synthesis. Refinement was brought to completion using anisotropic temperature factors for the non-hydrogen atoms and fixed B values, corresponding to those of the carrier atoms, for the hydrogens. The final R index is 0.058.[4] During the refinement the weight assigned to each observation was $w = (a + | F_0 | + c | F_0 |^2)^{-1}$ with $a = 3.0$ and $c = 0.007$, selected to keep a relatively constant average $\langle w | \Delta F |^2 \rangle$ in groups of F_0 and sin θ/λ (13). The atomic scattering factors were taken from the *International Tables for X-ray Crystallography* (14). All calculations were carried out on the UNIVAC 1110 computer of Rome University and were performed by a system of programs developed in this laboratory (15).

The atomic coordinates and temperature factors of tolypomycinone and of the water solvent molecule (Table 1) define, in a right-handed system, the absolute configuration of the molecule according to the determination by Kamiya *et al.* (10). The molecular structure of tolypomycinone is shown in Fig. 4.[5]

[4] A table listing the observed amplitudes and calculated structural factors is available on request from: The Director, CNR Laboratory of Structural Chemistry, P.O. Box No. 10, 00016 Monterotondo Stazione, Rome, Italy.

[5] The orientation of the molecule in Fig. 4 is different from the orientation used in Figs. 1–3; it was chosen in order to present a clearer view of the stereochemistry of the hydrogen atoms. For comparison of tolypomycinone with the ansamycins of Figs. 1–3, see Fig. 6.

Bond lengths and angles are given in Tables 2 and 3, respectively, except for hydrogen atoms. The mean distance of the 45 hydrogens from the parent atoms is 0.95(10) A, while the average values of bond angles involving hydrogens are 110(7)°, 117(6)°, and 107(8)° for C(sp^3), C(sp^2), and O carrier atoms, respectively. There are two intramolecular hydrogen bonds, i.e., O(1)—O(2) (2.55 A) and O(9)—O(10) (2.76 A), in which O(1) and O(9) are acceptors of the hydrogen atoms bound to O(2) and O(10), respectively. The solvent water molecule is involved in a network of multiple hydrogen bonds (Fig. 5), in which O(14) interacts with molecules of tolypomycinone related by a screw axis parallel to the crystal axis a. The two strongest hydrogen bonds occur between O(14) and O(9) (2.73 A) and between O(14) and O(8′) (2.90 A); in the first, O(14) is the acceptor of the hydrogen bound to O(9), while in the second, O(8′) is the acceptor for one of the hydrogen atoms of the water molecule. The second hydrogen atom of the solvent molecule is presumably engaged in a bifurcated hydrogen bond with O(8) (3.18 A) and O(6′) (3.16 A) of two different tolypomycinone molecules.

RESULTS AND DISCUSSION

The tolypomycinone molecule (Fig. 6) consists of a planar part, formed by the naphthoquinone nucleus and the 5-membered ring condensed to it, and a 17-membered chain that is connected to atoms C(2) and C(12). The distance of each atom from the best plane passing through the 10 atoms of the naphthoquinone ring is reported in Table 4. Oxygen atoms O(1) and O(2) and the amide nitrogen are also approximately in this plane, whereas the atoms of the 5-membered ring, C(11), C(12), O(3), and O(4), are slightly above this plane, shifted toward the ansa bridge. The plane of the amide group, N—C(15)—O(12)—C(16), is rotated 12° with respect to the plane of the naphthoquinone ring, with O(12) 2.85 A distant from C(3). The C(16)—C(17) bond is *cis*, and the plane containing the atoms of the ketonic group, C(17)—C(18)—C(19)—O(11), is rotated 26° with respect to the plane through C(15)—C(16)—C(17)—C(18)—C(30). Oxygen atom O(11) lies 3.28

TABLE 1

Fractional coordinates and thermal parameters[a] with their estimated standard deviations (in parentheses)

	x	y	z	B11	B12	B13	B22	B23	B33
C(1)	.2971(4)	-.0241(2)	.3444(2)	79(4)	-10(4)	-4(13)	23(1)	6(2)	17(1)
C(2)	.3002(4)	-.1101(2)	.3473(2)	76(4)	8(4)	-26(4)	22(1)	1(2)	24(1)
C(3)	.2013(5)	-.1501(2)	.3318(3)	94(4)	11(4)	-26(5)	19(1)	-2(2)	34(1)
C(4)	.0859(5)	-.1123(2)	.3150(2)	90(4)	-6(4)	-21(4)	23(1)	-8(2)	31(1)
C(5)	-.0302(4)	.0115(2)	.3193(2)	61(3)	-8(3)	-6(3)	19(1)	-2(2)	20(1)
C(6)	-.0300(4)	.0930(2)	.3225(2)	70(3)	-14(3)	-7(3)	21(1)	5(2)	15(1)
C(7)	.0717(4)	.1375(2)	.3297(2)	74(3)	-11(4)	1(3)	23(1)	9(2)	19(1)
C(8)	.1805(4)	.0971(2)	.3353(2)	67(3)	-5(3)	-5(3)	21(1)	2(2)	20(1)
C(9)	.1850(4)	.0153(2)	.3333(2)	54(3)	-9(3)	2(3)	22(1)	3(2)	19(1)
C(10)	.0776(4)	-.0269(2)	.3241(2)	77(4)	-7(3)	-8(3)	19(1)	-2(2)	17(1)
C(11)	-.1576(4)	-.0106(2)	.3187(2)	72(3)	-13(4)	-11(3)	25(1)	1(2)	20(1)
C(12)	-.2292(4)	.0666(2)	.3146(2)	62(3)	-16(4)	-18(3)	29(1)	5(2)	18(1)
C(13)	-.2961(5)	.0747(3)	.2454(2)	101(5)	-2(5)	-38(4)	39(2)	7(2)	23(1)
C(14)	.0692(5)	.2231(2)	.3333(3)	82(4)	-5(4)	-21(5)	20(1)	3(2)	38(2)
C(15)	.4426(5)	-.2154(2)	.3612(2)	92(4)	34(4)	-20(4)	26(1)	-12(2)	28(1)
C(16)	.5715(4)	-.2297(2)	.3836(2)	81(4)	7(4)	6(4)	20(1)	-1(2)	31(1)
C(17)	.6050(4)	-.2295(3)	.4506(3)	68(4)	25(4)	-2(4)	29(1)	-2(2)	30(2)
C(18)	.5237(4)	-.2132(2)	.5102(2)	64(3)	17(4)	10(4)	27(1)	5(2)	28(1)
C(19)	.5832(4)	-.1802(3)	.5732(2)	62(4)	27(4)	6(4)	34(2)	7(2)	27(1)
C(20)	.5376(4)	-.1013(3)	.5988(2)	62(3)	5(4)	-8(4)	31(1)	0(2)	26(1)
C(21)	.4383(4)	-.0603(2)	.5623(2)	55(3)	-4(3)	-4(3)	22(1)	-4(2)	22(1)
C(22)	.3971(4)	.0095(2)	.6055(2)	48(3)	-0(3)	-8(3)	30(1)	-16(2)	27(1)
C(23)	.2799(3)	.0456(2)	.5762(2)	47(3)	-8(3)	6(3)	26(1)	-3(2)	23(1)
C(24)	.1669(3)	-.0023(2)	.5832(2)	48(3)	7(3)	8(3)	22(1)	0(2)	23(1)
C(25)	.0596(3)	.0470(2)	.5595(2)	49(3)	4(3)	1(3)	20(1)	1(2)	18(1)
C(26)	-.0653(3)	.0089(2)	.5591(2)	45(3)	-2(3)	4(3)	27(1)	6(2)	20(1)
C(27)	-.1728(3)	.0581(2)	.5486(2)	43(3)	4(3)	-5(3)	30(1)	3(2)	18(1)
C(28)	-.1834(4)	.0768(2)	.4709(2)	55(3)	1(4)	-2(3)	30(1)	3(2)	20(1)
C(29)	-.2807(4)	.0580(2)	.4376(2)	50(3)	-13(3)	5(3)	29(1)	5(2)	22(1)
C(30)	.6558(6)	-.2439(3)	.3230(3)	112(5)	11(6)	31(5)	38(2)	2(3)	34(2)
C(31)	.5167(5)	-.1716(3)	.6416(2)	103(5)	24(5)	2(4)	39(2)	15(2)	23(1)
C(32)	.4912(5)	.0726(3)	.6109(4)	63(4)	-5(5)	-25(5)	41(2)	-37(4)	53(2)
C(33)	.1502(5)	-.0339(3)	.6574(3)	72(4)	11(5)	-4(4)	48(2)	40(3)	34(2)
C(34)	-.0690(4)	-.0668(2)	.5283(3)	75(4)	-13(4)	-16(4)	24(1)	6(2)	38(2)
C(35)	.0593(5)	.1843(3)	.5714(3)	65(4)	2(4)	8(5)	25(1)	-1(3)	48(2)
C(36)	.0660(9)	.2492(3)	.6245(5)	167(9)	0(7)	-16(11)	31(2)	-31(4)	73(4)
C(37)	-.1955(5)	.1260(4)	.6579(2)	80(4)	53(6)	-10(4)	68(3)	-21(3)	22(1)
N	.4114(4)	-.1391(2)	.3641(2)	86(4)	6(3)	-27(4)	23(1)	-5(2)	35(1)
O(1)	.3923(3)	.0107(2)	.3506(2)	59(2)	-4(3)	-3(3)	24(1)	7(2)	34(1)
O(2)	.2804(3)	.1380(2)	.3419(2)	73(3)	-17(3)	-12(3)	21(1)	9(2)	41(1)
O(3)	-.1392(3)	.1263(2)	.3192(2)	64(2)	8(2)	-5(4)	27(1)	-2(2)	31(1)
O(4)	-.2074(3)	-.0712(2)	.3220(2)	79(3)	-23(3)	-2(4)	27(1)	-2(2)	53(2)
O(5)	-.3133(3)	.0737(2)	.3684(2)	52(2)	5(3)	-20(2)	38(1)	0(2)	24(1)
O(6)	-.1802(3)	.1312(2)	.5840(2)	80(3)	27(3)	8(3)	35(1)	-10(2)	21(1)
O(7)	.0642(3)	.1159(2)	.6025(2)	59(2)	3(3)	-12(2)	23(1)	-7(1)	27(1)
O(8)	.0487(4)	.1938(2)	.5092(2)	116(4)	7(3)	24(5)	29(1)	26(2)	51(2)
O(9)	.2953(3)	.0653(2)	.5028(2)	59(2)	-5(3)	18(3)	28(1)	8(2)	27(1)
O(10)	.4809(3)	-.0385(2)	.4935(2)	73(3)	8(3)	10(3)	28(1)	4(2)	24(1)
O(11)	.4161(3)	-.2248(2)	.5060(2)	72(3)	-1(3)	5(3)	33(1)	-4(2)	39(1)
O(12)	.3808(4)	-.2661(2)	.3382(3)	118(4)	23(4)	-60(5)	30(1)	-34(2)	53(2)
O(13)	.0040(4)	-.1493(2)	.2923(3)	98(4)	-6(4)	-49(5)	28(1)	-27(3)	68(2)

[a] *The anisotropic temperature factors are defined as* $T = \exp[-10^{-4}(B_{11}h^2 + B_{12}hk + B_{13}hl + B_{22}k^2 + B_{23}kl + B_{33}l^2)]$.

A from O(12). The torsion angles around the bonds of the ansa bridge are shown in Table 5, in which the convention of Klyne and Prelog (16) has been adopted. Because of the cyclopropylic ring, the C(19)—C(20) bond has a fully eclipsed conformation, and this is also the region of sharpest bending of the chain. The points of minimum distance of the ansa bridge from the naphthoquinone ring occur at O(9) and O(10), which lie 2.49 and 2.90 A, respectively, from the plane of the ring. The best plane through the 17 atoms of the skeleton of the ansa bridge makes an angle of 61° with the plane of the naphthoquinone ring. With respect to the skeleton of the ansa bridge, the methyl group on C(22), the acetoxyl, and the methoxyl groups are on the same side of O(1) and O(2), and the amide oxygen O(12), the carbonyl oxygen O(11), and the

TABLE 1.—*Continued*

H(C3)	.2064(63)	-.2001(38)	.3231(32)	3.6 Å^2					
H(C13)	-.3546(61)	.0303(39)	.2566(35)	3.8					
H(C13)	-.3078(64)	.1289(39)	.2411(34)	3.8					
H(C13)	-.2405(64)	.0702(37)	.2019(35)	3.8					
H(C14)	.0967(68)	.2520(36)	.2887(36)	4.0					
H(C14)	-.0069(61)	.2361(39)	.3264(33)	4.0					
H(C14)	.1063(63)	.2455(38)	.3728(36)	4.0					
H(C17)	.6986(66)	-.2372(36)	.4595(33)	3.7					
H(C19)	.6641(60)	-.1907(36)	.5742(37)	3.5					
H(C20)	.5893(62)	-.0662(35)	.6172(36)	3.7					
H(C21)	.3803(57)	-.0939(32)	.5560(33)	2.8					
H(C22)	.3841(50)	-.0201(31)	.6561(30)	2.1					
H(C23)	.2695(58)	.0905(33)	.6070(32)	2.9					
H(C24)	.1723(55)	-.0474(34)	.5482(31)	2.8					
H(C25)	.0726(53)	.0628(31)	.5104(29)	2.4					
H(C26)	-.0841(57)	-.0067(31)	.6192(32)	2.8					
H(C27)	-.2404(57)	.0297(33)	.5645(31)	2.8					
H(C28)	-.1318(57)	.1176(33)	.4511(33)	3.1					
H(C29)	-.3430(56)	.0247(34)	.4545(33)	3.0					
H(C30)	.6655(69)	-.1991(45)	.2986(39)	5.0					
H(C30)	.6229(72)	-.2791(44)	.2964(40)	5.0					
H(C30)	.7343(70)	-.2737(42)	.3399(39)	5.0					
H(C31)	.4416(66)	-.1924(39)	.6369(33)	4.1					
H(C31)	.5594(68)	-.1873(41)	.6793(33)	4.1					
H(C32)	.5069(70)	.1038(41)	.5688(39)	4.8					
H(C32)	.5533(73)	.0535(42)	.6337(39)	4.8					
H(C32)	.4460(75)	.1162(43)	.6440(38)	4.8					
H(C33)	.2146(67)	-.0685(39)	.6707(36)	4.3					
H(C33)	.0855(69)	-.0651(38)	.6594(38)	4.3					
H(C33)	.1590(65)	.0061(41)	.6844(38)	4.3					
H(C34)	-.0396(62)	-.0555(36)	.4813(34)	3.7					
H(C34)	-.1557(60)	-.0824(36)	.5291(34)	3.7					
H(C34)	-.0039(60)	-.1086(37)	.5471(34)	3.7					
H(C36)	.0645(91)	.3002(48)	.5915(42)	7.0					
H(C36)	.1359(80)	.2367(51)	.6538(49)	7.0					
H(C36)	-.0181(81)	.2411(51)	.6503(47)	7.0					
H(C37)	-.2609(70)	.0847(43)	.6691(38)	4.8					
H(C37)	-.1344(68)	.1126(44)	.6815(38)	4.8					
H(C37)	-.2157(72)	.1716(45)	.6757(37)	4.8					
H(N1)	.4564(66)	-.1085(38)	.3881(35)	4.0					
H(O2)	.3524(53)	.1064(34)	.3449(31)	2.6					
H(O9)	.3018(61)	.1198(35)	.5000(33)	3.2					
H(O10)	.4390(62)	-.0115(33)	.4773(32)	3.3					
O(14)	.3295(4)	.2203(2)	.5121(3)	127(5)	-26(4)	9(6)	32(1)	24(3)	65(2)
H(O14)	.3933(74)	.2410(48)	.4959(48)	5.8					
H(O14)	.2942(80)	.2421(53)	.4964(52)	5.8					

methyl group on C(26) are on the opposite side. The hydroxyls on C(21) and C(23) and the methyl group on C(24) lie almost on the average plane of the ansa chain. The ansa bridge conformation in tolypomycinone differs considerably from the conformation found in rifamycin B *p*-iodoanilide and rifampicin. The major differences concern the positions of the two hydroxyls on C(21) and C(23) and that of the amidic CO group (Fig. 6).

In tolypomycinone the two hydroxyls point toward the naphthoquinone ring, while in rifamycin B *p*-iodoanilide (Fig. 2) and in rifampicin (Fig. 3) the C(21)—O and C(23)—O bonds are almost parallel to the plane of the naphthoquinone ring, and the O(9) and O(10) atoms are at a much greater distance from the phenolic hydroxyls (Table 6). This difference between the ansa chains of tolypomycinone and the rifamycins can be attributed to the difference in the chemical nature of the chain between C(18) and C(21). In particular, it can be attributed to the close contact between the ketonic O(11) and the amidic O(12). The segment from the amidic group to C(21) is sterically the most rigid part of the chain in both tolypomycinone and the rifamycins, and therefore a conformational switch is very difficult in this region. With regard to activity-conformation relationships in the naphthalenic ansamycins, it appears that the relative spatial positions of the O(1),

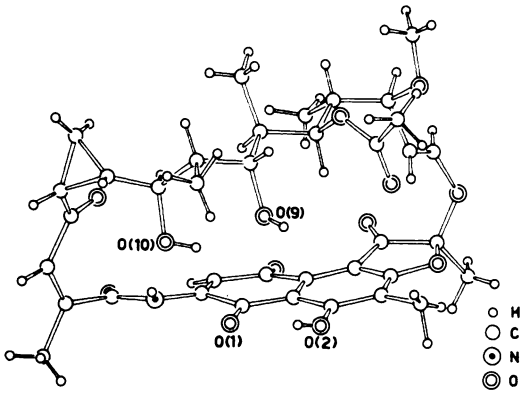


FIG. 4. *Molecular structure of tolypomycinone, showing also the positions of all hydrogen atoms*

O(2), O(9), and O(10) atoms are among the factors determining antibacterial activity. In fact, in the active ansamycins of known crystal structure (rifampicin and rifamycin B *p*-iodoanilide), the distances between the two hydroxyls on C(21) and C(23) and the phenolic hydroxyls are very close and are different from those in tolypomycinone (Table 6). On the basis of these findings, we suggest that the two pairs of hydroxyls form hydrogen bonds with a specific site on bacterial RNA polymerase. In both rifamycin S and the streptovaricins, the hydroxyls on C(1) and C(8) can be transformed into quinone groups without loss of activity; hence O(1) and O(2) are probably hydrogen bond acceptors. Tolypomycinone may be less active because the spatial positions of the hydroxyl groups on C(21) and C(23) are less suited to form hydrogen bonds with bacterial RNA polymerase. The stabilization of a conformation close to the optimal one for interaction with RNA polymerase would make complex formation with the enzyme more probable.

Tolypomycinone and the rifamycins also differ in the conformation of the amidic group. In the rifamycins the amidic oxygen lies on the same side of the phenolic hydroxyls on C(1) and C(8) (VII), while in tolypomycinone the amidic oxygen lies on the opposite side (VIII). A similar conformation has been found in tolypomycinone 8,21,23-tri-*m*-bromobenzoate (10), which, although devoid of activity because of the substituents, presents a conformation around C(21) and C(23) close to that suggested for activity (Table 6). Indeed, in tolypomycinone, the amidic plane forms an angle of 12° with the average plane of the naphthoquinone ring, and in its tri-*m*-bromobenzoate derivative this angle is 21°. Consequently 3-formyltolypomycinone derivatives, in contrast to the analogous rifamycin derivatives such as hydrazones and oximes, should have low activity, since the presence of the substituent at position 3 of the naphthoquinone ring should cause a large variation in the orientation of the amidic group, with probable drastic changes in the conformation of the ansa chain. In tolypomycinone, stabilization of the molecule in a conformation similar to the optimal one for antibacterial activity should instead be possible by introducing a group at position 3 that is able to form a hydrogen bond with the amidic oxygen. Support for this hypothesis derives from the recent report (6) that highly active derivatives are obtained by adding a primary

TABLE 2

Bond lengths with estimated standard deviations (in parentheses)

	A		*A*
C(1)—C(2)	1.498(6)	C(16)—C(30)	1.506(8)
C(1)—C(9)	1.444(6)	C(17)—C(18)	1.478(7)
C(1)—O(10)	1.230(6)	C(18)—C(19)	1.482(6)
C(2)—C(3)	1.339(7)	C(10)—O(11)	1.224(6)
C(2)—N	1.379(6)	C(19)—C(20)	1.542(7)
C(3)—C(4)	1.484(7)	C(19)—C(31)	1.508(7)
C(4)—C(10)	1.498(5)	C(20)—C(21)	1.489(6)
C(4)—O(13)	1.199(7)	C(21)—C(22)	1.535(6)
C(5)—C(6)	1.419(5)	C(21)—O(10)	1.439(5)
C(5)—C(10)	1.380(6)	C(22)—C(23)	1.556(6)
C(5)—C(11)	1.477(6)	C(22)—C(32)	1.524(7)
C(6)—C(7)	1.383(6)	C(23)—C(24)	1.520(6)
C(6)—O(3)	1.354(5)	C(23)—O(9)	1.443(5)
C(7)—C(8)	1.409(6)	C(24)—C(25)	1.541(5)
C(7)—C(14)	1.490(6)	C(24)—C(33)	1.521(7)
C(8)—C(9)	1.423(5)	C(25)—C(26)	1.556(6)
C(8)—O(2)	1.331(5)	C(25)—O(7)	1.450(5)
C(9)—C(10)	1.419(6)	C(26)—C(27)	1.526(5)
C(11)—C(12)	1.566(6)	C(26)—C(34)	1.528(6)
C(11)—O(4)	1.192(5)	C(27)—C(28)	1.513(5)
C(12)—C(13)	1.518(6)	C(27)—O(6)	1.441(5)
C(12)—O(3)	1.448(5)	C(28)—C(29)	1.299(6)
C(12)—O(5)	1.392(5)	C(29)—O(5)	1.390(5)
C(15)—C(16)	1.523(7)	C(35)—C(36)	1.515(9)
C(15)—N	1.372(6)	C(35)—O(7)	1.328(6)
C(15)—O(12)	1.203(6)	C(35)—O(8)	1.197(8)
C(16)—C(17)	1.325(7)	C(37)—O(6)	1.413(6)

TABLE 3

Bond angles with estimated standard deviations (in parentheses)

C(2)—C(1)—C(9)	120.0(4)°	C(16)—C(17)—C(18)	124.1(4)°
C(2)—C(1)—O(1)	122.1(4)°	C(17)—C(18)—C(19)	114.5(4)°
C(9)—C(1)—O(1)	117.9(4)°	C(17)—C(10)—O(11)	121.6(4)°
C(1)—C(2)—C(3)	119.4(4)°	C(19)—C(18)—O(11)	123.9(4)°
C(1)—C(2)—N	113.3(4)°	C(18)—C(19)—C(20)	116.7(4)°
C(3)—C(2)—N	127.3(4)°	C(18)—C(19)—C(32)	120.4(4)°
C(2)—C(3)—C(4)	122.5(4)°	C(20)—C(19)—C(32)	58.3(3)°
C(3)—C(4)—C(10)	117.9(4)°	C(19)—C(20)—C(21)	121.8(4)°
C(3)—C(4)—O(13)	120.2(4)°	C(19)—C(20)—C(31)	59.7(3)°
C(10)—C(4)—O(13)	121.8(5)°	C(21)—C(20)—C(31)	121.6(4)°
C(6)—C(5)—C(10)	118.6(4)°	C(20)—C(21)—C(22)	110.8(3)°
C(6)—C(5)—C(11)	105.2(3)°	C(20)—C(21)—O(10)	107.4(3)°
C(10)—C(5)—C(11)	135.8(3)°	C(22)—C(21)—O(10)	112.0(3)°
C(5)—C(6)—C(7)	124.4(4)°	C(21)—C(22)—C(23)	112.5(3)°
C(5)—C(6)—O(3)	115.1(4)°	C(21)—C(22)—C(32)	113.4(4)°
C(7)—C(6)—O(3)	120.5(3)°	C(23)—C(22)—C(32)	108.4(4)°
C(6)—C(7)—C(8)	116.0(3)°	C(22)—C(23)—C(24)	116.6(3)°
C(6)—C(7)—C(14)	123.2(4)°	C(22)—C(23)—O(9)	109.8(3)°
C(8)—C(7)—C(14)	120.8(4)°	C(24)—C(23)—O(9)	108.3(3)°
C(7)—C(8)—C(9)	121.8(4)°	C(23)—C(24)—C(25)	108.5(3)°
C(7)—C(8)—O(2)	117.7(4)°	C(23)—C(24)—C(33)	112.4(3)°
C(9)—C(8)—C(7)	121.8(4)°	C(25)—C(24)—C(33)	112.0(3)°
C(1)—C(9)—C(8)	120.1(4)°	C(24)—C(25)—C(26)	115.4(3)°
C(1)—C(9)—C(10)	120.5(3)°	C(24)—C(25)—O(7)	105.5(3)°
C(8)—C(9)—C(10)	119.3(4)°	C(26)—C(25)—O(7)	108.6(3)°
C(4)—C(10)—C(5)	121.7(4)°	C(25)—C(26)—C(27)	116.1(3)°
C(4)—C(10)—C(9)	118.3(4)°	C(25)—C(26)—C(34)	109.4(3)°
C(5)—C(10)—C(9)	119.8(3)°	C(27)—C(26)—C(34)	109.4(3)°
C(5)—C(11)—C(12)	105.7(3)°	C(26)—C(27)—C(28)	115.5(5)°
C(5)—C(11)—O(4)	132.9(4)°	C(26)—C(27)—O(6)	114.9(3)°
C(12)—C(11)—O(4)	121.4(3)°	C(28)—C(27)—O(6)	105.1(3)°
C(11)—C(12)—C(13)	112.0(3)°	C(27)—C(28)—C(29)	119.0(4)°
C(11)—C(12)—O(3)	104.9(3)°	C(28)—C(29)—O(5)	129.0(4)°
C(11)—C(12)—O(5)	112.7(3)°	C(19)—C(31)—C(20)	62.0(3)°
C(13)—C(12)—O(3)	109.1(3)°	C(36)—C(35)—O(7)	111.8(5)°
C(13)—C(12)—O(5)	106.9(4)°	C(36)—C(35)—O(8)	123.8(5)°
O(3)—C(12)—O(5)	111.3(3)°	O(7)—C(35)—O(8)	124.4(5)°
C(16)—C(15)—N	112.9(4)°	C(2)—N—C(15)	125.0(4)°
C(16)—C(15)—O(12)	121.6(4)°	C(6)—O(3)—C(12)	108.9(3)°
N—C(15)—O(12)	125.2(5)°	C(12)—O(5)—C(29)	119.8(3)°
C(15)—C(16)—C(17)	122.3(4)°	C(27)—O(6)—C(37)	114.4(4)°
C(15)—C(16)—C(31)	114.1(4)°	C(25)—O(7)—C(35)	119.3(4)°
C(17)—C(16)—C(31)	123.6(5)°		

amine in position 3 of tolypomycinone. In these derivatives, the formation of a hydrogen bond between the group introduced at position 3 and the amidic CO group is possible (IX).

Derivatives of this type show high activity while those obtained by adding secondary amines do not, since formation of the hydrogen bond is no longer possible. By contrast, very active derivatives can be obtained by adding secondary amines to position 3 of the naphthoquinone nucleus of rifamycins.

In rifampicin, according to Gadret *et al.* (11) and Ferrari and Gallo (17), a hydrogen bond is formed between the amidic NH group and the hydrazonic nitrogen. In fact, there is an angle of 41° between the amidic NH bond and a line connecting the two nitrogen atoms (between which the hydrogen bond should be formed), and this angle seems too large for hydrogen bond forma-

tion. When the amidic hydrogen atom, which is not well determined, is disregarded, an angle of 54° is found between a line connecting the two nitrogen atoms and the amidic plane in which the hydrogen atom should lie; this angle is far too large for a stable hydrogen bond. In 3-formylrifamycin hydrazones, the substituent probably presses on the amidic group without forming the hydrogen bond, and in the rifamycins substituents introduced on that site influence the position of the amidic group. Our findings are supported by the observation that the amidic plane and the average plane of the naphthoquinone ring form an angle of 26° in rifamycin B *p*-iodoanilide and an angle of 54°, much larger, in rifampicin. By contrast, the formation of a hydrogen bond between the amidic NH and the hydrazonic group, almost coplanar with the naphthoquinone ring, should have made the amidic group more coplanar with the naphthohydroquinone ring. The ab-

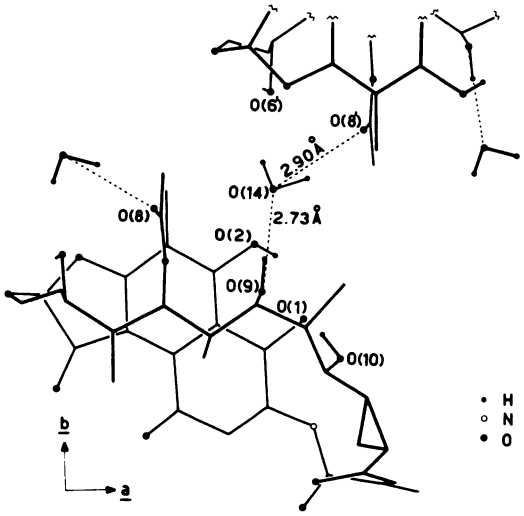


FIG. 5. *Intermolecular hydrogen bonds in crystal packing*

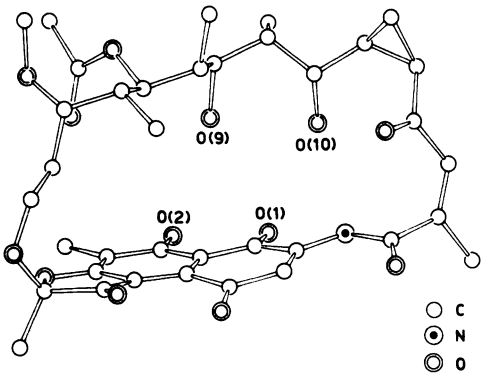


FIG. 6. *Molecular structure of tolypomycinone (non-hydrogen atoms only)*

TABLE 4

Distances of atoms of tolypomycinone from the best plane through C(1)-C(10)

	A		A		A
C(1)	0.00	C(18)	2.85	C(35)	4.51
C(2)	0.11	C(19)	3.90	C(36)	5.44
C(3)	0.02	C(20)	4.40	C(37)	6.60
C(4)	−0.13	C(21)	3.86	N	0.25
C(5)	0.06	C(22)	4.69	O(1)	−0.07
C(6)	0.06	C(23)	4.32	O(2)	−0.14
C(7)	−0.01	C(24)	4.68	O(3)	0.16
C(8)	−0.06	C(25)	4.38	O(4)	0.47
C(9)	−0.05	C(26)	4.80	O(5)	1.42
C(10)	0.00	C(27)	4.56	O(6)	5.19
C(11)	0.28	C(28)	3.11	O(7)	5.13
C(12)	0.28	C(29)	2.68	O(8)	3.35
C(13)	−0.91	C(30)	0.86	O(9)	2.90
C(14)	0.00	C(31)	5.29	O(10)	2.49
C(15)	0.20	C(32)	4.59	O(11)	2.96
C(16)	0.41	C(33)	6.14	O(12)	−0.09
C(17)	1.60	C(34)	4.09	O(13)	−0.39

TABLE 5

Torsion angles along the skeleton of the ansa bridge

The convention of Klyne and Prelog (16) has been adopted.

C(1)—C(2)—N—C(15)	−171°	C(21)—C(22)—C(23)—C(24)	68°
C(2)—N—C(15)—C(16)	179°	C(22)—C(23)—C(24)—C(25)	173°
N—C(15)—C(16)—C(17)	80°	C(23)—C(24)—C(25)—C(26)	−175°
C(15)—C(16)—C(17)—C(18)	−2°	C(24)—C(25)—C(26)—C(27)	178°
C(16)—C(17)—C(18)—C(19)	−152°	C(25)—C(26)—C(27)—C(28)	67°
C(17)—C(18)—C(19)—C(20)	122°	C(26)—C(27)—C(28)—C(29)	123°
C(18)—C(19)—C(20)—C(21)	0°	C(27)—C(28)—C(29)—O(5)	175°
C(19)—C(20)—C(21)—C(22)	170°	C(28)—C(29)—O(5)—C(12)	42°
C(20)—C(21)—C(22)—C(23)	−170°	C(29)—O(5)—C(12)—O(3)	70°

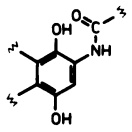
sence of this hydrogen bond can be explained in terms of intramolecular distances: in rifamycin B *p*-iodoanilide the distance between the phenolic oxygen O(1) and the amidic oxygen O(11) is 2.46 A, one of the smallest found for hydrogen bonds of the type OH—O. The formation of a hydrogen bond between the amidic NH and the hydrazonic group would have reduced the angle between the amidic plane and the naphthohydroquinone ring, thus reducing this distance even more and making it too small for an OH—O hydrogen bond. Steric hindrance thus prevails, and in rifampicin the O(1)—O(11) distance is 2.81 A.

We conclude that the substituents introduced at position 3 of both the naphthoquinone and naphthohydroquinone rings of rifamycins and tolypomycins play an important role in determining the most probable conformations of the antibiotics and their relative stability. This influence, according to our model, is translated into the activity of these antibiotics against bacterial RNA polymerase. It is possible to synthesize active tolypomycins by introducing groups, such as NH, that can form hydrogen bonds with the amidic CO. Active rifamycins can be obtained by introducing at the same position a group able to cause steric hindrance on the amidic NH. On the basis of this scheme, it is not easy to explain why tolypomycin Y is so much more active than tolypomycinone. Determination of the crystal structure of tolypomycin Y is under way, but so far none of the crystals has proved suitable for X-ray work.

TABLE 6

Distances between O(1), O(2), O(9), and O(10) in ansamycins

Oxygens	Rifamycin B *p*-iodoanilide	Rifampicin	Tolypomycinone tri-*m*-bromobenzoate	Tolypomycinone
	A	*A*	*A*	*A*
O(1)—O(2)	2.6	2.48	2.8	2.55
O(1)—O(9)	6.7	6.17	6.0	3.23
O(1)—O(10)	5.7	5.41	5.4	3.01
O(2)—O(9)	7.8	6.82	6.9	3.31
O(2)—O(10)	7.5	6.93	7.3	4.77
O(9)—O(10)	2.7	2.72	2.8	2.56



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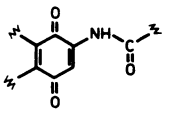
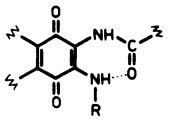


VIII



IX

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