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Structural Investigations of Mode of Action of Drugs. III.* Structure of Rifamycin S Iminomethyl Ether

BY S. K. ARORA

Department of Chemistry, The University of Arizona, Tucson, Arizona 85721, *USA*

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Abstract

A structural study of rifamycin S iminomethyl ether ($C_{38}H_{47}NO_{12}$) has been carried out by X-ray diffraction. The needle-shaped crystals belong to orthorhombic space group $P2_12_12_1$. The unit-cell dimensions are a = 14·059 (3), b = 21·420 (5) and c = 26·961 (5) Å. There are two molecules per asymmetric unit and eight molecules in the cell. The structure, with 102 nonhydrogen atoms in the asymmetric unit, was solved by repeated use of direct methods *via* tangent extension and refined by least-squares techniques to a final weighted residual of 0·109 for 4351 independent reflections. Attempts to locate H atoms were partially successful. The introduction of an extra double bond N=C(15) had a pronounced effect on the conformation of the ansa chain. The C(16)–C(17) and C(18)–C(19) double bonds are *cis* and *trans* respectively. The C(21)–O(10) and C(23)–O(9) bonds point toward the naphthoquinone ring rather than being parallel to it. A similar situation is observed in tolypomycinone and this seems to be the reason for the inactivity of both these compounds. There are four intramolecular and two intermolecular hydrogen bonds.

Introduction

The rifamycins are a group of antibiotics obtained by fermentation of *Streptomyces mediterranei* and chemical modification (Sensi, Maggi, Furesz & Maffii, 1966; Riva & Silvestri, 1972). They belong to the ansamycins, a name proposed by Prelog & Oppolzer (1973) for structures which include an aromatic moiety spanned by an aliphatic bridge. Rifamycin B is the major product under certain growth conditions; however, it is unstable and is oxidized in buffered neutral solutions by mild oxidizing agents, or even in air, to rifamycin O with the loss of two H atoms. Rifamycin S is obtained by hydrolysis from rifamycin O and rifamycin SV by reduction from rifamycin S. The structures of rifamycins B, O, S, SV were elucidated by chemical degradation (Prelog & Oppolzer, 1973; Oppolzer, Prelog & Sensi, 1964) and by X-ray studies on rifamycin B *p*-iodoanilide (Brufani, Fedeli, Giacomello & Vaciago, 1964). The activity of rifamycins against Gram-positive and Gram-negative bacteria is due to the inhibition of RNA synthesis catalyzed by bacterial DNA-dependent RNA polymerase (Hartman, Honikel, Knusel & Nuesch, 1967; Mizuno, Yamazaki, Nitta & Umezawa, 1968). The various actions of the rifamycins have been reviewed by Wherli & Staehelin (1971).

The rifamycin molecule has been extensively modified chemically. Most of the modifications have consisted of substitutions in positions 3 and/or 4 of the naphthoquinone chromophore. These modifications had pronounced effects on the *in vivo* action of the drug. Rifampicin, a semisynthetic derivative and the most active, is the best example. Chemical modification of the ansa bridge in general reduces the capacity of the substance to inhibit enzyme activity, to form a stable complex with the enzyme, and to affect bacterial growth. It seems that the antibacterial activity of this class of antibiotics is closely connected with the

* Part I: Arora (1979*a*). Part II: Arora (1979*b*).

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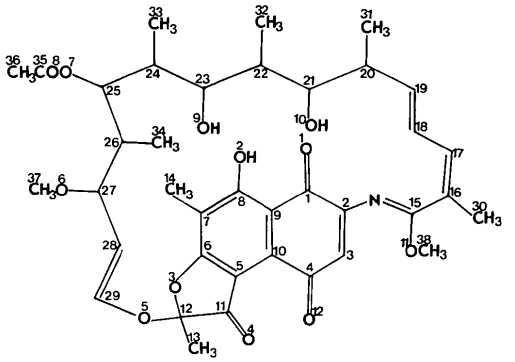

Fig. 1. Rifamycin S iminomethyl ether showing the numbering scheme.

presence of (*a*) a naphthalene ring carrying two O atoms [either in quinone form or as free hydroxyl groups at C(1) and C(8)] and (*b*) two free hydroxyl groups at C(21) and C(23). Conformation/activity studies have been carried out on rifamycins B, Y (Brufani, Cerrini, Fedeli & Vaciago, 1974), rifampicin (Gadret, Goursolle, Leger & Colleter, 1975) and tolypomycinone (Brufani, Cellai, Cerrini, Fedeli & Vaciago, 1978). To investigate further the relationship between the conformation of the ansa chain and the activity or inactivity of the ansamycins, X-ray studies were carried out on the iminomethyl ether derivative of rifamycin S (Fig. 1).

Experimental section

Red needle-shaped crystals were obtained by slow evaporation from ether of a sample kindly provided by Dr J. McCarthy of Dow Leptit. Preliminary Weissenberg photographs indicated that the crystals were orthorhombic with space group $P2_12_12_1$. A crystal of dimensions 0·2 × 0·2 × 0·3 mm was used for the measurement of cell constants and data collection. The crystal data are presented in Table 1. The cell parameters were obtained by least-squares fittings of the settings of four angles of 13 reflections on a Picker FACS-I diffractometer. The intensity data were collected using a scintillation counter with a pulse-height analyzer, θ–2θ scan technique, 2° min^{-1} scan rate, 10 s background counts, attenuators when count rate exceeded 10^4 counts s^{-1}, and a 2° scan range with a dispersion factor allowing for α_1–α_2 splitting at large 2θ values. Of the 6620 independent reflections collected, 4351 had intensities $\geq 3\sigma(I)$ and were considered observed ($2\theta \leq 110°$) and used in the analysis. Three standard reflections were monitored every 50 measurements to check crystal alignment and stability. Lorentz and polarization corrections were applied to the data but no correction was made for absorption.

Table 1. *Crystal data for rifamycin S imino ethyl ether*

Empirical formula	$C_{38}H_{47}NO_{12}$	V	8119·13 Å^3
M_r	709·3	ρ_c	1·314 Mg m^{-3}
Space group	$P2_12_12_1$	Z	8
a	14·059 (3) Å	Radiation	Cu $K\alpha$ (λ = 1·54178 Å)
b	21·420 (5)		
c	26·961 (5)		

Structure determination and refinement

Attempts to solve the structure (102 atoms/asymmetric unit) by the direct-method program *MULTAN* (Germain, Main & Woolfson, 1971) met with failure initially. The program had difficulty in picking origin reflections.

h	k	l	E	φ	
0	1	8	3·54	$\pi/2$	(origin)
11	9	9	2·41	$\pi/4$	(origin)
9	11	6	2·85	$\pi/4$	(origin, enantiomorph)
9	12	1	2·71	$\pm\pi/4, \pm 3\pi/4$	
1	1	7	2·45	$\pm\pi/4, \pm 3\pi/4$	
1	1	5	2·35	$\pm\pi/4, \pm 3\pi/4$	

After many attempts at fixing the origin manually in *MULTAN* (Germain, Main & Woolfson, 1971) one starting set with 300 E's > 1·85 produced an E map in which a ten-atom fragment made good chemical and geometrical sense ($R_{\text{Karle}} = 0{\cdot}46$). In all the manual origin-fixing attempts that failed, the combined figures of merit (CFOM) were very low (<2·0), even after varying the ratio NSRT (number of Σ_2 relationships)/NUMB (number of E's). However, in the successful attempt, by variation of NSRT/NUMB from 4·0 to 6·0, the highest CFOM rose from 1·91 to 2·40. The ten-atom fragment revealed a 20-atom fragment *via* tangent extension. Further application of the tangent-extension method six times and picking atoms which made good chemical sense revealed 80 nonhydrogen atoms. The last 22 atoms were located by the difference Fourier method. This probably is the second-largest structure (102 nonhydrogen atoms) without a heavy atom which has been solved by direct methods.

Two cycles of full-matrix least-squares refinement with individual isotropic thermal parameters reduced R from 0·38 to 0·147. The refinement was carried out in parts because of the large number of variables. Two more cycles of refinement of the nonhydrogen atoms with anisotropic thermal parameters reduced R to 0·109 for 4351 observed reflections. Attempts to locate H atoms in the difference Fourier map were only partially successful and so they were not included in the refinement. The refinement, based on F_o, the quantity minimized being $\sum w(|F_o| - |F_c|)^2$, was terminated at this stage since the shifts were all less than one-third of an e.s.d. The weighting scheme used was based on counter statistics as defined by Corfield, Doedens &

Ibers (1967); the value of p was 0·04. The scattering factors used were those of Hanson, Herman, Lea & Skillman (1964).* No correction was applied for primary extinction but a few reflections which were affected by secondary extinction were not included in the final cycles of refinement.

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35592 (29 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Results and discussion

The atomic coordinates and the temperature factors for the two independent molecules (I and II) are given in

Table 2. *Fractional coordinates* ($\times 10^4$) *and* B_{eq} (Å^2)

The atoms of molecule (II) are denoted by asterisks. $B_{eq} = 8\pi^2(U_{11}\,U_{22}\,U_{33})^{1/3}$, where U_{11}, U_{22}, U_{33} are the amplitudes along the principal axes of the thermal ellipsoid.

	x	y	z	B_{eq}		x	y	z	B_{eq}
O(1)	5060 (7)	3696 (4)	2294 (3)	4·88	O(1)*	−2119 (8)	958 (6)	544 (4)	8·44
O(2)	6470 (5)	4440 (3)	2319 (2)	4·94	O(2)*	−445 (7)	1188 (4)	854 (3)	9·90
O(3)	5933 (5)	6526 (3)	1804 (2)	4·88	O(3)*	704 (5)	98 (3)	2220 (2)	6·98
O(4)	3441 (4)	6605 (3)	1610 (2)	4·22	O(4)*	−984 (6)	−1071 (3)	2476 (3)	4·12
O(5)	5231 (5)	7092 (2)	1164 (2)	4·58	O(5)*	430 (5)	−324 (2)	3031 (2)	6·00
O(6)	4110 (8)	6368 (4)	−310 (3)	4·33	O(6)*	−1295 (6)	382 (3)	4356 (2)	4·06
O(7)	7522 (6)	6004 (4)	−519 (3)	8·25	O(7)*	641 (5)	2117 (3)	3827 (2)	5·76
O(8)	8428 (15)	6532 (10)	−39 (7)	8·11	O(8)*	1812 (6)	1396 (4)	3866 (2)	4·35
O(9)	6811 (4)	5578 (2)	813 (1)	17·06	O(9)*	227 (3)	1546 (2)	2630 (1)	6·04
O(10)	5543 (5)	4665 (3)	1003 (2)	5·47	O(10)*	−1517 (4)	1272 (2)	2245 (2)	4·73
O(11)	2242 (4)	2589 (2)	2016 (2)	6·72	O(11)*	−5340 (5)	642 (4)	473 (2)	4·88
O(12)	2641 (4)	5554 (2)	2077 (2)	5·94	O(12)*	−2302 (7)	−1070 (4)	1700 (3)	8·50
N	3225 (5)	3400 (3)	2155 (3)	4·64	N*	−3826 (7)	368 (5)	622 (3)	2·08
C(1)	4545 (9)	4157 (5)	2164 (4)	3·72	C(1)*	−2210 (10)	511 (8)	871 (4)	6·20
C(2)	3484 (9)	4005 (5)	2091 (4)	3·47	C(2)*	−3117 (9)	141 (8)	927 (5)	5·61
C(3)	2904 (9)	4489 (6)	1997 (4)	3·93	C(3)*	−3107 (10)	−353 (8)	1210 (6)	6·73
C(4)	3212 (8)	5135 (5)	2034 (4)	3·27	C(4)*	−2329 (9)	−542 (9)	1527 (5)	6·07
C(5)	4653 (7)	5873 (4)	1914 (3)	2·52	C(5)*	−739 (7)	−209 (4)	1881 (3)	3·60
C(6)	5613 (5)	5941 (3)	1934 (2)	2·77	C(6)*	84 (6)	201 (3)	1870 (2)	3·71
C(7)	6253 (8)	5497 (4)	2098 (3)	2·82	C(7)*	212 (9)	667 (6)	1523 (4)	4·84
C(8)	5872 (8)	4909 (4)	2184 (3)	2·29	C(8)*	−516 (8)	738 (5)	1187 (4)	4·58
C(9)	4875 (8)	4768 (4)	2140 (3)	2·75	C(9)*	−1380 (8)	362 (6)	1209 (4)	4·91
C(10)	4249 (8)	5263 (4)	2027 (3)	2·93	C(10)*	−1447 (8)	−121 (5)	1552 (4)	4·54
C(11)	4252 (8)	6451 (4)	1731 (4)	3·22	C(11)*	−532 (8)	−626 (5)	2289 (4)	4·14
C(12)	5076 (9)	6900 (5)	1650 (4)	3·68	C(12)*	417 (7)	−452 (4)	2501 (4)	3·91
C(13)	5039 (9)	7471 (5)	1977 (4)	4·26	C(13)*	1192 (10)	−954 (5)	2438 (4)	5·63
C(14)	7298 (9)	5623 (6)	2157 (4)	4·16	C(14)*	1131 (9)	1052 (5)	1523 (4)	4·96
C(15)	2702 (6)	3098 (3)	1861 (3)	4·50	C(15)*	−4649 (7)	488 (5)	792 (3)	6·26
C(16)	2449 (7)	3239 (5)	1338 (3)	5·40	C(16)*	−4998 (7)	528 (5)	1316 (3)	5·94
C(17)	3110 (7)	3374 (5)	991 (4)	5·46	C(17)*	−4583 (7)	911 (4)	1652 (3)	5·32
C(18)	4133 (8)	3364 (5)	1053 (4)	5·96	C(18)*	−3798 (6)	1331 (4)	1588 (2)	4·57
C(19)	4674 (9)	3562 (5)	685 (4)	6·79	C(19)*	−3413 (6)	1617 (4)	1972 (3)	4·80
C(20)	5754 (9)	3633 (5)	718 (4)	6·78	C(20)*	−2578 (6)	2088 (4)	1955 (3)	4·68
C(21)	6004 (6)	4315 (4)	619 (3)	4·73	C(21)*	−1873 (5)	1880 (3)	2359 (2)	3·45
C(22)	7088 (6)	4458 (5)	577 (4)	4·77	C(22)*	−992 (7)	2317 (4)	2411 (3)	3·40
C(23)	7204 (5)	5187 (4)	426 (2)	4·42	C(23)*	−298 (5)	2086 (3)	2799 (2)	3·27
C(24)	6746 (7)	5339 (4)	−70 (3)	4·33	C(24)*	−769 (7)	1935 (4)	3308 (3)	3·68
C(25)	6702 (8)	6051 (5)	−190 (3)	5·17	C(25)*	−46 (8)	1630 (4)	3656 (3)	4·23
C(26)	5828 (9)	6256 (5)	−423 (3)	5·08	C(26)*	−378 (8)	1244 (5)	4112 (4)	4·46
C(27)	5008 (8)	6248 (4)	−35 (3)	4·51	C(27)*	−918 (7)	685 (4)	3925 (3)	3·69
C(28)	5086 (9)	6766 (5)	338 (3)	5·62	C(28)*	−210 (8)	237 (4)	3670 (3)	4·56
C(29)	5179 (7)	6645 (4)	808 (3)	4·44	C(29)*	−279 (7)	76 (4)	3189 (3)	3·75
C(30)	1360 (8)	3228 (5)	1206 (4)	6·80	C(30)*	−5886 (10)	115 (7)	1414 (5)	8·82
C(31)	6181 (13)	3218 (6)	317 (6)	9·85	C(31)*	−2932 (8)	2762 (4)	2070 (3)	5·75
C(32)	7603 (9)	4339 (5)	1075 (4)	6·19	C(32)*	−518 (8)	2433 (5)	1922 (4)	4·68
C(33)	7250 (10)	4937 (5)	−500 (4)	6·92	C(33)*	−1206 (9)	2539 (5)	3515 (4)	5·74
C(34)	5512 (10)	5940 (5)	−913 (3)	6·05	C(34)*	−1059 (10)	1622 (5)	4479 (4)	5·94
C(35)	8108 (12)	6574 (9)	−430 (5)	6·58	C(35)*	1542 (7)	1938 (5)	3896 (4)	9·33
C(36)	8811 (12)	6689 (7)	−830 (6)	9·13	C(36)*	2153 (10)	2462 (7)	4023 (5)	4·01
C(37)	3299 (12)	6212 (9)	−33 (5)	10·60	C(37)*	−2009 (11)	−59 (6)	4245 (5)	7·55
C(38)	2304 (8)	2452 (6)	2531 (4)	9·70	C(38)*	−5116 (14)	707 (11)	−68 (5)	6·87

Table 3. *Bond distances* (Å)

	(I)	(II)		(I)	(II)		(I)	(II)
C(1)–C(2)	1·54 (2)	1·51 (2)	C(11)–C(12)	1·52 (2)	1·50 (2)	C(22)–C(32)	1·54 (2)	1·50 (1)
C(1)–C(9)	1·39 (2)	1·51 (2)	C(11)–O(4)	1·23 (1)	1·25 (2)	C(23)–C(24)	1·52 (1)	1·56 (1)
C(1)–O(1)	1·27 (2)	1·31 (2)	C(12)–C(13)	1·51 (2)	1·54 (2)	C(23)–O(9)	1·45 (1)	1·45 (1)
C(2)–C(3)	1·34 (2)	1·31 (2)	C(12)–O(3)	1·51 (2)	1·46 (1)	C(24)–C(25)	1·56 (2)	1·53 (2)
C(2)–N	1·36 (1)	1·38 (2)	C(12)–O(5)	1·39 (1)	1·46 (1)	C(24)–C(33)	1·61 (2)	1·53 (2)
C(3)–C(4)	1·46 (2)	1·45 (2)	C(15)–N	1·26 (1)	1·27 (1)	C(25)–C(26)	1·45 (2)	1·55 (2)
C(4)–C(10)	1·48 (2)	1·53 (2)	C(15)–C(16)	1·48 (1)	1·50 (1)	C(25)–O(7)	1·44 (1)	1·50 (2)
C(4)–O(12)	1·21 (1)	1·22 (2)	C(15)–O(11)	1·34 (1)	1·34 (1)	C(26)–C(27)	1·56 (2)	1·51 (1)
C(5)–C(6)	1·36 (1)	1·45 (1)	C(16)–C(17)	1·35 (2)	1·36 (1)	C(26)–C(34)	1·55 (2)	1·60 (2)
C(5)–C(10)	1·46 (1)	1·35 (2)	C(16)–C(30)	1·57 (2)	1·55 (2)	C(27)–C(28)	1·50 (1)	1·54 (2)
C(5)–C(11)	1·45 (1)	1·45 (2)	C(17)–C(18)	1·45 (2)	1·43 (1)	C(27)–O(6)	1·49 (2)	1·43 (1)
C(6)–C(7)	1·38 (1)	1·38 (1)	C(18)–C(19)	1·32 (2)	1·32 (1)	C(28)–C(29)	1·30 (1)	1·35 (1)
C(6)–O(3)	1·38 (1)	1·30 (1)	C(19)–C(20)	1·53 (2)	1·55 (1)	C(29)–O(5)	1·36 (1)	1·38 (1)
C(7)–C(8)	1·39 (1)	1·38 (2)	C(20)–C(21)	1·53 (2)	1·54 (1)	C(35)–O(7)	1·22 (1)	1·34 (1)
C(7)–C(14)	1·50 (2)	1·53 (2)	C(20)–C(31)	1·52 (2)	1·56 (1)	C(35)–C(36)	1·49 (2)	1·45 (2)
C(8)–C(9)	1·44 (2)	1·46 (2)	C(21)–C(22)	1·56 (1)	1·56 (1)	C(35)–O(8)	1·15 (2)	1·22 (1)
C(8)–O(2)	1·36 (1)	1·32 (2)	C(21)–O(10)	1·43 (1)	1·43 (1)	C(37)–O(6)	1·40 (2)	1·41 (2)
C(9)–C(10)	1·41 (2)	1·39 (2)	C(22)–C(23)	1·62 (1)	1·52 (1)	C(38)–O(11)	1·42 (1)	1·50 (2)

Table 4. *Bond angles* (°)

	(I)	(II)		(I)	(II)		(I)	(II)
C(2)–C(1)–C(9)	121·1 (9)	118·8 (9)	C(5)–C(10)–C(9)	118·4 (8)	119·5 (8)	C(23)–C(22)–C(32)	109·3 (7)	112·0 (7)
C(2)–C(1)–O(1)	114·9 (9)	122·3 (9)	C(5)–C(11)–C(12)	106·7 (8)	108·4 (7)	C(22)–C(23)–C(24)	112·6 (6)	113·7 (6)
C(9)–C(1)–O(1)	123·6 (9)	118·9 (9)	C(5)–C(11)–O(4)	132·9 (8)	132·4 (8)	C(22)–C(23)–O(9)	109·7 (6)	112·0 (5)
C(1)–C(2)–C(3)	116·7 (9)	118·3 (9)	C(12)–C(11)–O(4)	119·9 (8)	119·2 (7)	C(24)–C(23)–O(9)	110·4 (6)	109·1 (5)
C(1)–C(2)–N	116·5 (8)	111·4 (9)	C(11)–C(12)–C(13)	113·7 (9)	114·5 (8)	C(23)–C(24)–C(25)	114·1 (7)	110·3 (7)
C(3)–C(2)–N	126·7 (9)	130·0 (9)	C(11)–C(12)–O(3)	103·5 (8)	104·5 (7)	C(23)–C(24)–C(33)	109·4 (7)	108·4 (7)
C(2)–C(3)–C(4)	122·7 (9)	125·5 (9)	C(11)–C(12)–O(5)	116·1 (8)	115·6 (7)	C(25)–C(24)–C(33)	113·0 (8)	113·6 (8)
C(3)–C(4)–C(10)	117·9 (9)	118·2 (9)	C(13)–C(12)–O(3)	107·4 (8)	108·2 (7)	C(24)–C(25)–C(26)	114·8 (8)	120·9 (8)
C(3)–C(4)–O(12)	120·9 (8)	120·5 (9)	C(13)–C(12)–O(5)	108·5 (8)	103·3 (6)	C(24)–C(25)–O(7)	108·7 (8)	108·7 (7)
C(10)–C(4)–O(12)	121·1 (8)	120·1 (9)	O(3)–C(12)–O(5)	107·0 (7)	110·7 (6)	C(26)–C(25)–O(7)	107·9 (8)	108·7 (7)
C(6)–C(5)–C(10)	118·4 (8)	119·4 (7)	C(16)–C(15)–N	129·3 (6)	130·5 (7)	C(25)–C(26)–C(27)	109·5 (9)	108·0 (8)
C(6)–C(5)–C(11)	108·0 (7)	103·2 (7)	C(16)–C(15)–O(11)	110·5 (6)	110·8 (7)	C(25)–C(26)–C(34)	118·7 (9)	113·6 (9)
C(10)–C(5)–C(11)	133·3 (8)	137·4 (8)	N–C(15)–O(11)	120·2 (6)	118·6 (7)	C(27)–C(26)–C(34)	110·8 (9)	108·0 (8)
C(5)–C(6)–C(7)	125·8 (7)	123·7 (7)	C(15)–C(16)–C(17)	122·5 (7)	121·7 (7)	C(26)–C(27)–C(28)	112·8 (9)	108·6 (8)
C(5)–C(6)–O(3)	114·3 (6)	114·6 (6)	C(15)–C(16)–C(30)	116·4 (7)	113·0 (8)	C(26)–C(27)–O(6)	107·0 (8)	105·9 (7)
C(7)–C(6)–O(3)	119·7 (6)	121·6 (6)	C(17)–C(16)–C(30)	121·1 (8)	125·3 (6)	C(28)–C(27)–O(6)	105·6 (8)	108·5 (7)
C(6)–C(7)–C(8)	115·3 (7)	115·3 (8)	C(16)–C(17)–C(18)	126·8 (8)	129·1 (7)	C(27)–C(28)–C(29)	120·8 (9)	122·8 (8)
C(6)–C(7)–C(14)	123·1 (8)	119·9 (8)	C(17)–C(18)–C(19)	118·6 (8)	120·8 (7)	C(28)–C(29)–O(5)	123·6 (8)	113·8 (7)
C(8)–C(7)–C(14)	121·5 (8)	124·7 (9)	C(18)–C(19)–C(20)	124·1 (9)	126·3 (7)	C(36)–C(35)–O(7)	112·6 (9)	111·8 (7)
C(7)–C(8)–C(9)	123·5 (8)	122·3 (9)	C(19)–C(20)–C(21)	108·3 (8)	106·2 (6)	C(36)–C(35)–O(8)	115·0 (9)	124·3 (8)
C(7)–C(8)–O(2)	118·5 (8)	118·1 (8)	C(19)–C(20)–C(31)	106·9 (9)	110·9 (7)	O(7)–C(35)–O(8)	107·4 (9)	123·8 (7)
C(9)–C(8)–O(2)	118·0 (8)	119·6 (8)	C(21)–C(20)–C(31)	110·2 (9)	109·4 (6)	C(2)–N–C(15)	124·5 (8)	120·9 (7)
C(1)–C(9)–C(8)	121·2 (8)	120·2 (9)	C(20)–C(21)–C(22)	115·2 (7)	113·7 (6)	C(6)–O(3)–C(12)	107·1 (6)	109·1 (5)
C(1)–C(9)–C(10)	120·6 (8)	120·3 (9)	C(20)–C(21)–O(10)	105·7 (7)	109·7 (6)	C(12)–O(5)–C(29)	116·7 (6)	114·2 (6)
C(8)–C(9)–C(10)	117·9 (8)	119·5 (8)	C(22)–C(21)–O(10)	113·1 (6)	106·8 (6)	C(27)–O(6)–C(37)	112·6 (9)	113·2 (8)
C(4)–C(10)–C(5)	123·5 (8)	123·0 (8)	C(21)–C(22)–C(23)	107·8 (6)	112·3 (6)	C(25)–O(7)–C(35)	106·4 (8)	117·1 (6)
C(4)–C(10)–C(9)	118·1 (8)	117·5 (8)	C(21)–C(22)–C(32)	111·3 (7)	112·0 (7)	C(15)–O(11)–C(38)	116·5 (6)	119·7 (8)

Table 2. The stereochemistry of rifamycin S iminomethyl ether is shown in Fig. 2. The bond lengths and bond angles are given in Tables 3 and 4. There is good agreement between molecules (I) and (II) except occasional differences which may be due to errors in the data.

Both independent molecules have similar conformations and consist of a near-planar part, formed by the naphthoquinone nucleus and the five-membered ring condensed to it, and a 17-membered ansa chain that is connected to C(2) and C(12). The deviations of the nearby atoms from the least-squares plane through atoms C(1) to C(10) are given in Table 5. O(1), O(2) and the imine N atom are approximately in this plane, whereas atoms of the five-membered ring, C(11), C(12), O(3) and O(4), are slightly above the plane. The plane of the imine group N–C(15)–O(11)–C(16), makes an angle of 61·1 (0·8) in molecule (I) and 57·4 (0·7)° in molecule (II) with respect to the plane of the naphthoquinone ring. The N=C(15) bond has a *cis*

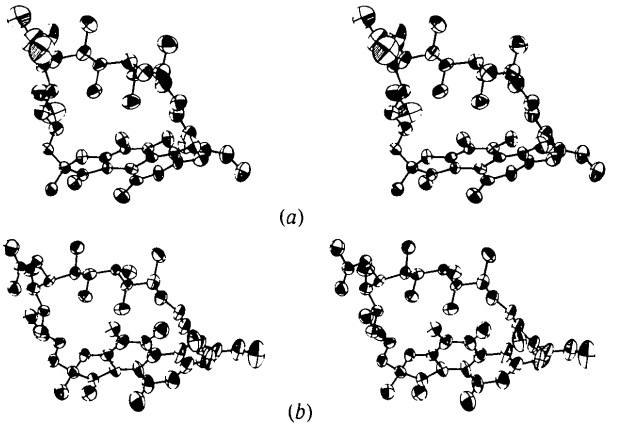

Fig. 2. Stereoscopic views of (*a*) molecule (I) and (*b*) molecule (II).

Table 5. *Least-squares planes through atoms* C(1) *to* C(10)

The equations for the planes are: molecule (I): $0{\cdot}113X - 0{\cdot}172Y - 0{\cdot}978Z = -6{\cdot}554$; molecule (II): $0{\cdot}407X - 0{\cdot}614Y - 0{\cdot}675Z = -3{\cdot}521$.

	(I)	(II)		(I)	(II)
C(1)	0·03 (1) Å	0·00 (1) Å	C(13)	−0·62 (1) Å	1·02 (1) Å
C(2)	0·11 (1)	0·14 (1)	C(14)	0·05 (1)	0·01 (1)
C(3)	0·08 (1)	0·00 (1)	C(15)	0·93 (1)	−1·22 (1)
C(4)	−0·20 (1)	0·12 (1)	C(38)	−0·66 (1)	−0·21 (1)
C(5)	0·07 (1)	−0·05 (1)	N	0·13 (1)	−0·29 (1)
C(6)	0·14 (1)	−0·09 (1)	O(1)	−0·06 (1)	0·05 (1)
C(7)	−0·02 (1)	−0·01 (1)	O(2)	−0·17 (1)	0·15 (1)
C(8)	−0·08 (1)	0·09 (1)	O(3)	0·32 (1)	0·24 (1)
C(9)	−0·07 (1)	0·05 (1)	O(4)	0·41 (1)	−0·14 (1)
C(10)	−0·06 (1)	0·03 (1)	O(5)	1·69 (1)	−1·32 (1)
C(11)	0·28 (1)	−0·13 (1)	O(11)	0·63 (1)	1·24 (1)
C(12)	0·45 (1)	−0·19 (1)	O(12)	−0·55 (1)	0·51 (1)

configuration. The C(16)–C(17) and C(18)–C(19) double bonds are *cis* and *trans* respectively and their conformation is *transoid* with respect to the C(17)–C(18) single bond. The C(28)–C(29) double bond has a *trans* configuration. The C(16)–C(17) bond has an almost eclipsed conformation. The torsion angles around the bonds of the ansa bridge are given in Table 6, which also contains torsion angles in tolypomycinone (Brufani *et al.*, 1978), rifampicin (Gadret *et al.*, 1975), rifamycin B *p*-iodoanilide and rifamycin Y *p*-iodoanilide (Brufani *et al.*, 1964). The convention of Klyne & Prelog (1960) has been adopted.

The best plane through the 17 atoms of the skeleton of the ansa bridge makes angles of 33·2 (0·7) and 30·7 (0·8)° (molecules I and II) with the plane of the naphthoquinone ring. These values differ from those in rifamycin B *p*-iodoanilide, 71·5 (0·8)°, and tolypomycinone, 61·8 (0·7)°. The introduction of an extra double bond [N=C(15)] has a pronounced effect on the conformation of the ansa bridge as compared to those

Table 6. *Torsion angles* (°) *along the skeleton of the ansa bridge of rifamycin S iminomethyl ether* (I *and* II), *tolypomycinone* (III), *rifampicin* (IV), *rifamycin B* (V) *and rifamycin Y* (VI)

The e.s.d.'s for molecules (I) and (II) are ~1°.

	(I)	(II)	(III)	(IV)	(V)	(VI)
C(1)–C(2)–N–C(15)	−132	−129	−171	−55	−32	−26
C(2)–N–C(15)–C(16)	17	11	179	179	180	168
N–C(15)–C(16)–C(17)	50	55	80	−30	−43	−47
C(15)–C(16)–C(17)–C(18)	4	0	−2	4	5	−8
C(16)–C(17)–C(18)–C(19)	−174	−172	−152	154	168	180
C(17)–C(18)–C(19)–C(20)	174	−179	122	−165	−175	171
C(18)–C(19)–C(20)–C(21)	−120	−132	0	−19	−11	111
C(19)–C(20)–C(21)–C(22)	−173	−178	170	169	170	−76
C(20)–C(21)–C(22)–C(23)	175	−178	−170	−176	−179	−84
C(21)–C(22)–C(23)–C(24)	−60	−51	68	62	53	143
C(22)–C(23)–C(24)–C(25)	170	173	173	165	174	180
C(23)–C(24)–C(25)–C(26)	−140	−160	−175	159	155	159
C(24)–C(25)–C(26)–C(27)	72	64	178	153	174	151
C(25)–C(26)–C(27)–C(28)	72	70	67	−171	−170	−174
C(26)–C(27)–C(28)–C(29)	−119	−119	123	118	117	113
C(27)–C(28)–C(29)–O(5)	−178	179	175	−175	−168	−176
C(28)–C(29)–O(5)–C(12)	163	−174	42	65	49	78
C(29)–O(5)–C(12)–O(3)	71	70	−70	−78	−68	−84

of rifamycin B *p*-iodoanilide and rifampicin, the major difference being in the spatial positions of the hydroxyls on C(21), C(23) and the imino group. In rifamycin S iminomethyl ether the two hydroxyls O(9) and O(10) point toward the naphthoquinone ring (as also observed in tolypomycinone), while in rifamycin B *p*-iodoanilide and rifampicin the C(21)–O(10) and C(23)–O(9) bonds are almost parallel to the plane of the naphthoquinone ring (Fig. 3). Rifamycin B *p*-iodoanilide and rifampicin are both very active while rifamycin S iminomethyl ether and tolypomycinone have very little activity. In active rifamycins, the hydroxyls O(1), O(2), O(9) and O(10) form hydrogen bonds with a specific site on bacterial RNA polymerase. O(1) and O(2) can be carbonyls without loss of activity. The spatial positions of O(1), O(2), O(9) and O(10) are the features determining the activity of rifamycins, as suggested by Brufani *et al.* (1978). Our study with the space-filling models indicates that active rifamycins prefer to attach to the β-sheet rather than to the α-helix (due to steric requirements) part of RNA polymerase through hydrogen bonds and stacking interactions (between the aromatic amino acid and the naphthoquinone ring).

Fig. 4 shows the packing of the molecules in the unit cell. Each molecule is involved in two intramolecular and one intermolecular hydrogen bond. Table 7 gives the hydrogen-bond distances and the atoms involved. The two intramolecular hydrogen bonds are similar to those in rifamycin B *p*-iodoanilide and tolypomycinone. Table 8 gives the distances between O(1), O(2), O(9) and O(10) for rifamycin S iminomethyl ether and related compounds. The distances between O(9) and O(10) on the ansa chain and O(1) and O(2) on the

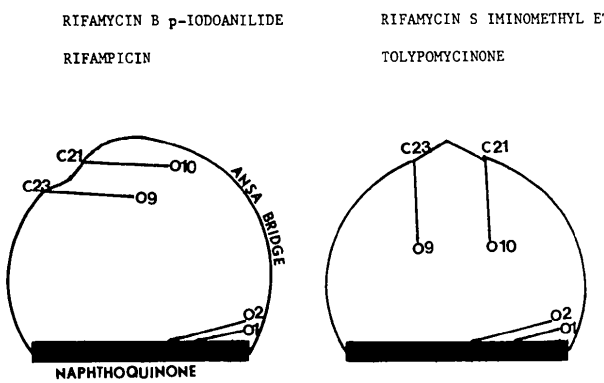

Fig. 3. Spatial arrangements of O(1), O(2), O(9) and O(10) in different rifamycins.

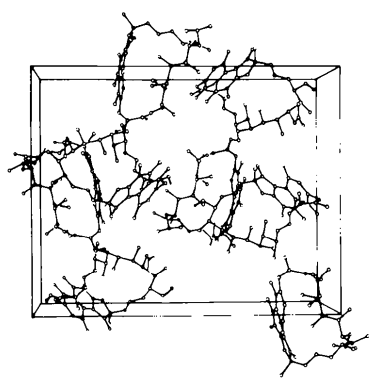

Fig. 4. A view of the unit cell projected down *a*.

Table 7. *Hydrogen bonds*

Asterisks indicate atoms of molecule (II)

D–H···*A*†	Position of *A*	*D*···*A* (Å)
O(2)–H···O(1)	x,y,z	2·544 (5)
O(9)–H···O(8)*	$1-x,\frac{1}{2}+y,\frac{1}{2}-z$	2·750 (6)
O(10)–H···O(9)	x,y,z	2·695 (5)
O(2)*–H···O(1)*	x,y,z	2·545 (5)
O(9)*–H···O(10)*	x,y,z	2·728 (6)
O(10)*–H···O(12)	$-x,-\frac{1}{2}+y,\frac{1}{2}-z$	2·864 (5)

† Donor–hydrogen···acceptor, *D*–H at x,y,z.

naphthoquinone ring are much larger in rifampicin and rifamycin B *p*-iodoanilide than those in tolypomycinone and rifamycin S iminomethyl ether. These distances have to be large for the stacking to occur between the naphthoquinone rings and aromatic amino acids.

Table 8. *Distances* (Å) *between* O(1), O(2), O(9) *and* O(10) *in ansamycins*

	Rifamycin S iminomethyl ether		Rifamycin B *p*-iodoanilide	Rifampicin	Tolypomycinone
O(1)–O(2)	2·54 (2)	2·55 (2)	2·6	2·48	2·55
O(1)–O(9)	6·18 (2)	6·64 (2)	6·7	6·17	3·23
O(1)–O(10)	4·11 (2)	4·70 (2)	5·7	5·41	3·01
O(2)–O(9)	4·76 (2)	4·94 (2)	7·8	6·82	3·31
O(2)–O(10)	3·81 (2)	4·06 (2)	7·5	6·93	4·77
O(9)–O(10)	2·70 (2)	2·77 (2)	2·7	2·72	2·56

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