Entstehung des Tetrahydropyranringes unter dem Einfluss von Säuren, weist auf eine Allylstellung des Methoxyls am C-27 hin, obwohl C-23 nicht ganz ausgeschlossen werden kann.

Die Rifamycine B, O, S und SV sind nach den vorgeschlagenen Formeln I bis IV natürliche ansa-Verbindungen[12], in welchen der aromatische Teil durch eine aliphatische Kette so überbrückt ist, dass diese durch die Ebene des aromatischen Systems nicht durchschwingen kann. Die Kette liegt deshalb fast senkrecht darauf im magnetischen Felde seiner π-Elektronen, was im NMR aus einer auffallenden Verschiebung der Doublette zweier CH_3CH-Gruppen ersichtlich ist (im Rifamycin S 0,64 und 0,22). Viel stärker als bei chinoiden Verbindungen ist die Verschiebung bei den entsprechenden Hydrochinonderivaten z.B. in der Verbindung XXVII (0,5 und −0,6). Durch methanolytische Spaltung des grossen Ringes erhält man aus der Verbindung XXVII die Verbindung XXVIII. Alle vier CH_3CH-Doublette der letzteren befinden sich erwartungsgemäss in einem dichten Signalhaufen, dessen Schwerpunkt bei 1 liegt.

Auf Grund der Konstitution I lässt sich vermuten, dass das Rifamycin B zum grossen Teil, ebenso wie viele andere Stoffwechselprodukte der Mikroorganismen aus biogenetischen Äquivalenten der Essigsäure und der Propionsäure aufgebaut ist. Die Konstitution der aliphatischen Brücke erinnert an diejenige gewisser Makrolide. Trotz dieser biogenetischen Verwandtschaft stellen Rifamycine eine strukturell neuartige Gruppe von Naturstoffen dar und geben ein weiteres Zeugnis von der Fähigkeit der Mikroorganismen, ungewöhnliche Verbindungen und besonders solche mit vielgliedrigen Ringen zu synthetisieren.

Wie aus der nachfolgenden Mitteilung von BRUFANI, FEDELI, GIACOMELLO und VACIAGO[13] folgt, konnte die auf Grund des chemischen Abbaus und der spektroskopischen Untersuchungen abgeleitete Konstitution I durch die Röntgenstrukturanalyse des Rifamycin B-*p*-jodanilids voll bestätigt werden.

Summary. Constitution I, based on extensive degradation studies, is proposed for Rifamycin B, a metabolite of *Streptomyces mediterraneus* n. sp.

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[12] A. LÜTTRINGHAUS und H. GRALHEER, Liebigs Ann. *550*, 67 (1941).
[13] M. BRUFANI, W. FEDELI, G. GIACOMELLO und A. VACIAGO, Exper. *20*, 339 (1964).

The X-Ray Analysis of the Structure of Rifamycin B[1]

The crystal structure of *p*-iodoanilide of rifamycin B has been elucidated in our laboratory in order to obtain an independent determination of the molecular structure of the antibiotic compound rifamycin B, $C_{39}H_{49}NO_{14}$, a product of the metabolism of *Streptomyces mediterranei* n. sp. (see preceding paper).

Crystallographic measurements were started in 1962 when knowledge of the chemical structure was still slight. However, enough was then known to prepare a variety of heavy-atom derivatives of rifamycin B and other related rifamycins. A preliminary study of these derivatives[3] indicated that the *p*-iodoanilide of rifamycin B, $C_{45}H_{53}N_2O_{13}I$, was the most promising for X-ray analysis. In fact, crystals of this compound, grown from a water-acetone solution and kept in a sealed capillary tube in the presence of mother liquor, were fairly stable and suitable for our measurements.

Crystal data: $C_{45}H_{53}N_2O_{13}I + C_3H_6O + 5H_2O$, $M = 956.83 + 58.08 + 90.08 = 1104.99$, Orthorombic disphenoidal, $a = 9.06 \pm 0.03$, $b = 23.66 \pm 0.08$, $c = 25.45 \pm 0.09$ Å, $U = 5456$ Å^3, $D_m = 1.23$ gcm^{-3} (by flotation), $Z = 4$, $D_c = 1.34^5$ gcm^{-3} (taking solvent molecules into account), $F(000) = 1976 + 128 + 200 = 2304$. Space group, $P2_12_12_1$ (D_2^4, No. 19). CuKα-radiation, $\mu = 53.2^5$ cm^{-1}, single crystal oscillation and Weissenberg photographs.

2175 reflections for intensity measurements were measured by eye from photographic records made by Weissenberg cameras at room temperature, using specimens measuring about $1.0 \cdot 0.3 \cdot 0.3$ mm and rotating around the *a* (6 layers) and *c* (6 layers) axes. They amounted to about 33% of the reflections theoretically possible in the CuKα sphere. Even the best crystals were of medium reflecting power and deteriorated noticeably in the X-ray beam; no reflections were observed beyond a spacing limit of 0.83 Å around the *c* axis and of 1.00 Å (with an average cut-off at about 1.2 Å) around the *a* axis. Absorption corrections are small and were not applied.

The iodine atom co-ordinates were determined without ambiguity from a three-dimensional unsharpened Patterson synthesis; a section of the Patterson function at $U = {}^1/_2$ is shown in Figure 1.

With phase angles calculated from these coordinates[4] ($x/a = 0.548$, $y/b = 0.087$, $z/c = 0.491$, $R = 41\%$), a three-dimensional Fourier synthesis was evaluated, including at this early stage all the structure-amplitudes without any weighing scheme. 61 maxima were interpreted as carbon atoms, giving an *R* value of 36%, but no information on the chemical structure could safely be given by the electron distribution at this stage. 17 maxima out of the 61 proved later to be spurious.

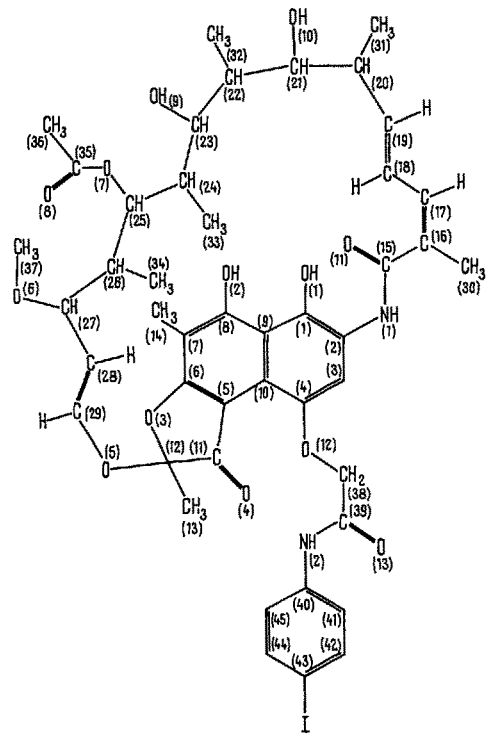
The elucidation of the structure continued by Fourier methods: the benzene ring of the anilide could be clearly

[1] A brief preliminary report on this structure has been read at a meeting of the Accademia Nazionale dei Lincei, Rome, on December 14, 1963[2].
[2] M. BRUFANI, W. FEDELI, G. GIACOMELLO, and A. VACIAGO, Rend. Acc. Lincei, *36*, 113 (1964).
[3] M. BRUFANI, W. FEDELI, G. GIACOMELLO, and A. VACIAGO, Chemotherapia (Basel) *7*, 145 (1963).
[4] It is noteworthy that the iodine atom coordinates from the Patterson function did not change much during refinement (see Table), and that they were such that there was no 'mirror image' problem.

seen on the second Fourier ($R = 33\%$) and the naphtoquinonic ring, with a large part of the rest of the molecule, on the fourth Fourier ($R = 27.3\%$). The fifth Fourier synthesis could be interpreted in terms of the 61 atoms of the whole molecule and of 10 other atoms of the solvent molecules (one of acetone and six of water), giving an R value of 24.2%. One spurious peak was still being interpreted as a real atom (a water molecule) at this stage. An overall B value of 5 Å^2 had been used up to this point.

From the seventh Fourier and a difference Fourier with the same phase angles, we derived the present structure. This is summarized in the Table, where the 61 atoms of the molecule and the other 9 atoms, interpreted as one molecule of acetone and five water molecules, are listed. Individual isotropic B values now range from about 3.3 to about 5.5 Å^2. The atomic coordinates of the Table give an R value of 20.2% and define bond lengths which deviate by a maximum of 0.4 Å (in one case) and by an average of 0.08 Å from acceptable values for formula (I). Such deviations are only to be expected at the present stage of structure refinement, because errors and uncertainties of a few tenths of an Ångstrom may well exist in some parts of the field. However, it seems unlikely that some small modification of the atomic positions during further refinement could be sufficient to change our interpretation of the chemical structure of the molecule.

Structure (I) can therefore be considered as established unambiguously, and a stereo-model, built from our crystallographic evidence, is given in Figure 2, showing



I

Atomic coordinates

Atom	x/a	y/b	z/c	Atom	x/a	y/b	z/c
C(1)	0.2425	0.0950	0.2862	C(37)	0.7782	0.3492	0.1850
C(2)	0.3131	0.0483	0.2734	C(38)	0.7120	0.0043	0.3371
C(3)	0.4540	0.0340	0.2936	C(39)	0.8697	0.0140	0.3658
C(4)	0.5455	0.0768	0.3181	C(40)	0.0771	0.0550	0.4031
C(5)	0.5583	0.1732	0.3568	C(41)	0.1830	0.0112	0.4068
C(6)	0.4644	0.2246	0.3692	C(42)	0.3132	0.0180	0.4345
C(7)	0.3405	0.2361	0.3518	C(43)	0.3375	0.0676	0.4622
C(8)	0.2553	0.1922	0.3283	C(44)	0.2367	0.1088	0.4587
C(9)	0.3343	0.1417	0.3127	C(45)	0.1022	0.1082	0.4261
C(10)	0.4648	0.1290	0.3336	N(1)	0.2520	0.0025	0.2438
C(11)	0.7188	0.1829	0.3763	N(2)	0.9426	0.0467	0.3792
C(12)	0.7015	0.2476	0.3907	O(1)	0.1025	0.1141	0.2658
C(13)	0.7417	0.2490	0.4536	O(2)	0.1142	0.2050	0.3096
C(14)	0.2567	0.2902	0.3609	O(3)	0.5542	0.2677	0.3890
C(15)	0.9058	0.4912	0.2624	O(4)	0.8130	0.1602	0.3683
C(16)	0.9325	0.4443	0.2994	O(5)	0.8095	0.2806	0.3663
C(17)	0.8667	0.4345	0.3476	O(6)	0.6913	0.2989	0.1837
C(18)	0.7575	0.4617	0.3713	O(7)	0.8913	0.1707	0.1586
C(19)	0.6695	0.4441	0.4135	O(8)	0.1253	0.1839	0.1450
C(20)	0.5464	0.4661	0.4379	O(9)	0.5709	0.1499	0.0608
C(21)	0.4944	0.0267	0.0799	O(10)	0.3724	0.0644	0.0665
C(22)	0.6306	0.0496	0.0587	O(11)	0.9947	0.0187	0.2596
C(23)	0.6831	0.1088	0.0786	O(12)	0.6575	0.0641	0.3416
C(24)	0.6736	0.1133	0.1400	O(13)	0.0646	0.4535	0.1366
C(25)	0.7033	0.1789	0.1540	I	0.5461	0.0889	0.4917
C(26)	0.6702	0.2007	0.2063				
C(27)	0.7417	0.2538	0.2205				
C(28)	0.7011	0.2759	0.2794	C(46)	0.4270	0.2922	0.0588
C(29)	0.8117	0.2762	0.3109	C(47)	0.5309	0.3483	0 0529
C(30)	0.0580	0.4117	0.2808	C(48)	0.7033	0.3505	0.0219
C(31)	0.4220	0.4214	0.4333	O(14)	0.5292	0.3900	0.0911
C(32)	0.1609	0.4482	0.0010	O(15)	0.5132	0.4243	0.2878
C(33)	0.7652	0.0676	0.1688	O(16)	0.4133	0.3505	0.2207
C(34)	0.4738	0.2041	0.2064	O(17)	0.2276	0.3529	0.1316
C(35)	0.9715	0.1985	0.1221	O(18)	0.0870	0.3083	0.0395
C(36)	0.9416	0.2244	0.0880	O(19)	0.0181	0.0677	0.0462

the relative configuration of the asymmetric carbon atoms and many other configurational and conformational details, as for instance the transoid conformation of the C(16)—C(17) and C(18)—C(19) double bonds across the C(17)—C(18) single bond. The electron density distribution from a Fourier synthesis phased on the positions

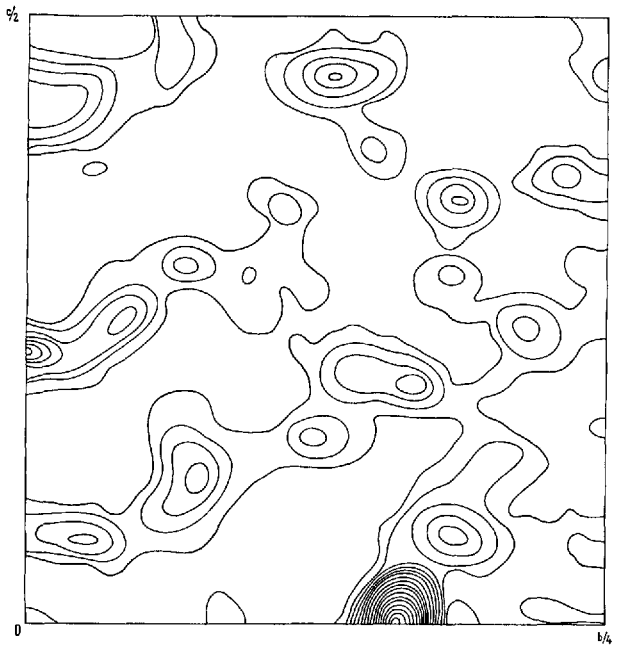


Fig. 1. Harker section at $U = {}^1/_2$.

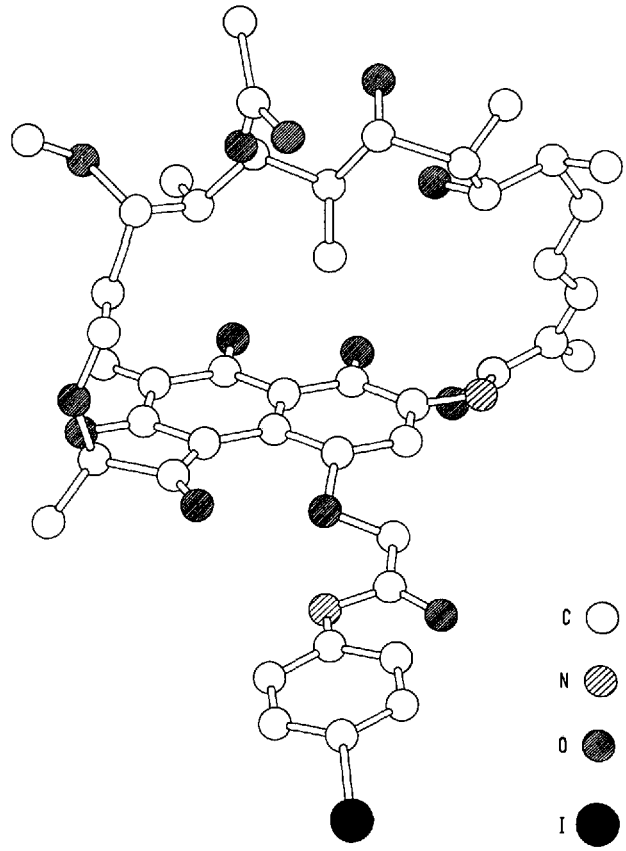


Fig. 2. Stereo-model of the structure of *p*-iodoanilide of rifamycin B from X-ray analysis evidence.

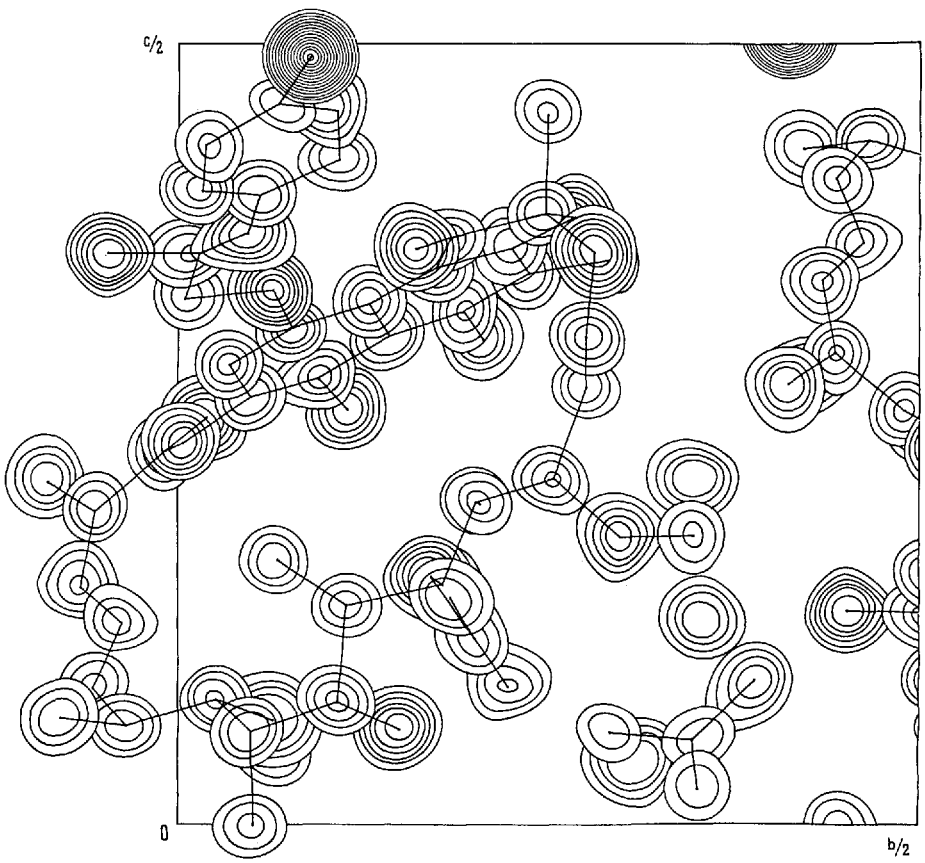


Fig. 3. The eighth three-dimensional electron density distribution shown by means of superimposed contour section projected on (100).

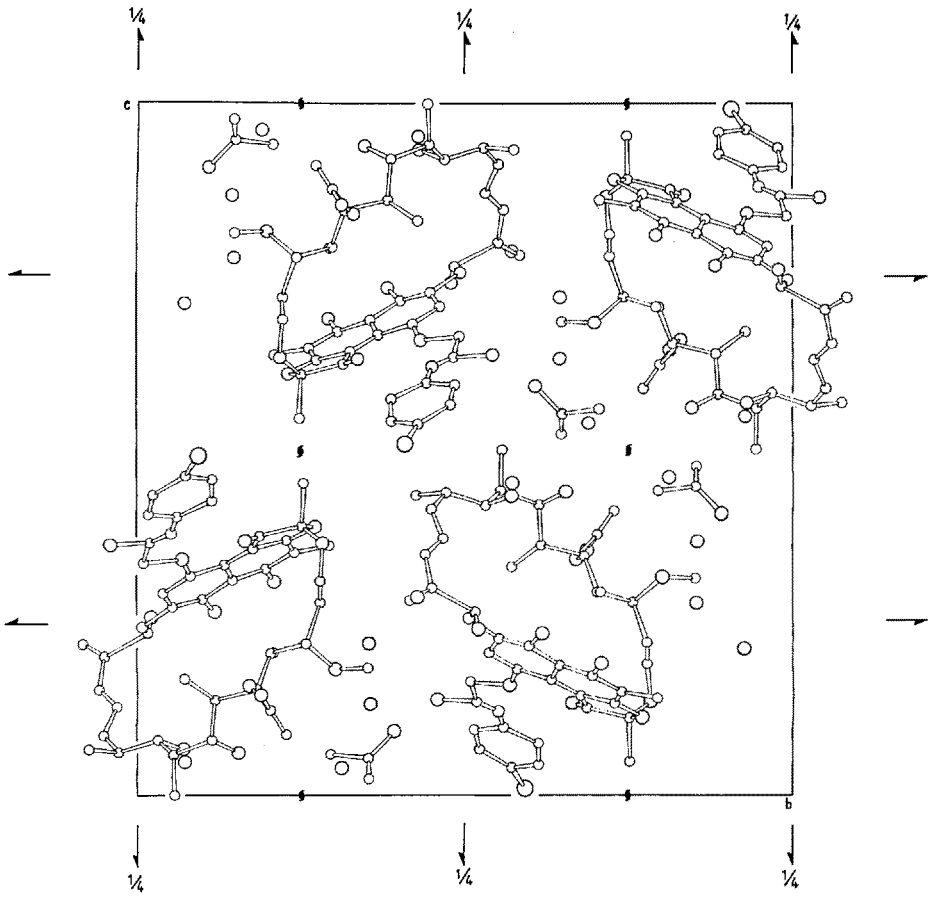


Fig. 4. Packing of the structure seen down the *a* axis. Space group $P2_12_12_1$.

of the Table is shown by means of superimposed contour sections in Figure 3. Figure 4 gives some idea of the mutual arrangement of the molecules in the unit cell.

Structure (I) is fully consistent with the independent chemical results on the constitution of rifamycin B obtained by OPPOLZER, PRELOG and SENSI (see preceding paper). Moreover, this chemical evidence, lately made available to us[5], speeded up the interpretation of the various Fourier syntheses considerably. On two issues this was especially true. First, at the present stage of refinement it would not have been possible to distinguish crystallographically beyond all doubt between oxygen, nitrogen, and carbon atoms, even if, on average, electron densities around these atomic positions came out in the correct decreasing order of magnitude. Second, the formulation of C(16)–C(17), C(18)–C(19) and C(28)–C(29) as double bonds and of the other C–C bonds in the aliphatic chain as single bonds, even though supported by various stereochemical features in the X-ray structure, as for instance the near planarity of the groups of atoms concerned with the double bonds, was also based on the chemical evidence available, and not on bond lengths which are of no significance for this purpose at the present stage. The hydrogen atoms given in structure (I) are, of course, based on chemical evidence only. On the other hand, X-ray analysis results, besides confirming the chemical ones, add a wealth of new stereochemical information.

A complete description of the structure of rifamycin B, with a full record of the bond lengths and bond angles in the molecule, will be given after further refinement has been carried as far as possible. This refinement will also make use of a few hundred new reflections, with lower *d*-limit, which are now being recorded from some very good crystals[6].

Zusammenfassung. Die molekulare Struktur des Rifamycins B wurde durch Röntgen-Strukturanalyse der Kristalle seines *p*-Jodanilids bestimmt. Die Ergebnisse bestätigen die vor kurzem auf chemischem Wege aufgeklärte Konstitution und liefern darüber hinaus interessante Auskunft über die Konfiguration und Konformation der Rifamycine.

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[5] V. PRELOG, Chemotherapia (Basel) *7*, 133 (1963).

[6] We are grateful to Professor P. SENSI of Lepetit SpA Research Laboratories, Milano, for making available supplies of the *p*-iodoanilide of rifamycin B and to Professor DOROTHY CROWFOOT HODGKIN, F.R.S. for helpful advice at the beginning of this research and for friendly interest throughout. We also thank Mr. A. TAMBURRINI and Mr. N. OCELLO for technical assistance. – All the calculations were carried out on the Rome University Science Faculty IBM 1620 computer.