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

One of the products of the metabolism of *Streptomyces mediterranei* is rifamycin Y, a compound chemically and physically similar to the antibiotic rifamycin B, $C_{39}H_{49}NO_{14}$, and yet biologically inactive[3]. Apart from this qualitative information, little was known at the outset about the structure of rifamycin Y. No explanation was therefore possible for the difference in the activity of the two compounds and for the even vaster difference in the activity of their respective derivatives.

In order to determine the constitution and the complete three-dimensional structure of rifamycin Y, and to compare it with that of rifamycin B, which had already been studied both by chemical[4-6] and crystallographic[7-9] methods, the crystal structure of the *p*-iodoanilide derivative of rifamycin Y was elucidated in our laboratory. The constitution and, in part, the configuration of rifamycin Y was also determined by LEITICH, PRELOG and SENSI (see preceding paper[10]) by chemical and spectroscopic methods. We are now very pleased to find that the independent results of the 2 groups agree completely.

In the case of rifamycin B the *p*-iodoanilide derivative had proved to be the most successful for X-ray analysis[7], and what was known of the chemical properties of rifamycin Y was enough to make us assume that this would also be the case for rifamycin Y. For this reason and because of the very small quantities available, no detailed study of various heavy-atom derivatives of rifamycin Y was performed. Crystals of the *p*-iodoanilide of rifamycin Y, grown from a water-dimethylsulphoxide solution and kept in a sealed capillary tube in the presence of mother liquor, even if of lower reflecting power than those of the same derivative of rifamycin B, were in fact stable enough to make a certain amount of data collection possible.

Crystal data[11]: $C_{45}H_{51}N_2O_{14}I + (CH_3)_2SO + H_2O$, $M = 970.82 + 78.13 + 18.02 = 1066.97$, monoclinic sphenoidal, $a = 9.49 \pm 0.03$, $b = 18.96 \pm 0.06$, $c = 15.30 \pm 0.05$ Å, $\beta = 90°48' \pm 30'$, $U = 2752.66$ Å^3, $D_c = 1.287$ gcm^{-3} for $Z = 2$ and taking solvent molecules into account[12], $F(000) = 1000 + 84 + 20 = 1104$. Space group $P2_1$ (C_2^2, No. 4). CuKα-radiation (λ taken as 1.5418 Å), $\mu = 55.4$ cm^{-1}, single crystal oscillation and Weissenberg photographs.

1426 reflections[13] for intensity measurements were measured by eye from photographic records made by Weissenberg cameras at room temperature, rotating around the *a* axis (6 layers, specimens of 0.12 · 0.24 and 0.04 · 0.07 mm cross section) and the *b* axis (8 layers, specimens of 0.06 · 0.25 and 0.08 · 0.33 mm cross section). The crystals were of low reflecting power and deteriorated noticeably in the X-ray beam. The cut-off for the majority of reflections on both axes was at a θ value of about 45°, corresponding to a spacing limit of about 1.1 Å and implying a resolution limit of approximately 0.65 Å, but a few reflections were observed at a maximum θ value as high as 48° around the *a* axis, and 53° around the *b* axis. An even more striking indication of the very poor quality of the data is given by the ratio between the number of reflections observed with intensity above film background and that of the reflections theoretically possible in the CuKα sphere (23%), as compared with the same ratio for the already difficult case of rifamycin B (33%).

Absorption corrections are small and were not applied, nor were the extinction corrections.

Because the space group $P2_1$ is polar, the origin must be defined with respect to the *b* direction. The *b* axis origin was arbitrarily chosen, for this purpose, as the midpoint between the 2 iodine atoms related by the two-fold screw axis, thus setting the *y* coordinate of 1 iodine atom at $y/b = 0.25$ and making the origin a pseudo-centre of inversion for the 2 related iodine atoms.

The *x* and *z* coordinates of the iodine atom were then determined from a three-dimensional Patterson synthesis sharpened on iodine. The parameters of the iodine atom gave a *R* value of 44.3%, and a Fourier synthesis was then computed, phased on this atom alone and therefore showing a pseudo-mirror plane at $y/b = 0.25$. This resulted in the superposition of the molecule onto its mirror image, causing duplication of the atoms in the cell. It was necessary to remove this ambiguity in order to isolate and identify the atoms corresponding to a single molecule[14].

This was done by the following procedure. On the basis of the known general similarity in the UV-spectra and chemical properties of rifamycins B and Y, it was assumed that they had the same planar parts (the naphthalene-like ring and the five-membered ring fused to it). The electron density map was then successfully searched for 2 consistent sets of atoms that could be interpreted accordingly, and for 2 *p*-iodoaniline rings in the correct positions relative to the other planar parts. It was further assumed that the structure was the same also in the vicinity of the planar parts, and as a result, of the 2 specular sets of atoms, the one which would lead to the same planar chirality of rifamycin B was chosen as the initial part of the 'real' molecule of rifamycin Y. This assumption was justified, post factum, by the circular dichroism measurements described in the preceding paper[10].

[1] A brief preliminary report on this structure was read at a meeting of the Accademia Nazionale dei Lincei, Rome, on 12th March 1966[2].

[2] M. BRUFANI, W. FEDELI, G. GIACOMELLO and A. VACIAGO, Atti Accad. naz. Lincei Rc. *40*, 548 (1966).

[3] P. SENSI, personal communication.

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

[5] W. OPPOLZER, V. PRELOG and P. SENSI, Experientia *20*, 336 (1964).

[6] J. LEITICH, W. OPPOLZER and V. PRELOG, Experientia *20*, 343 (1964).

[7] M. BRUFANI, W. FEDELI, G. GIACOMELLO and A. VACIAGO, Chemotherapia *7*, 145 (1963).

[8] M. BRUFANI, W. FEDELI, G. GIACOMELLO and A. VACIAGO, Atti Accad. naz. Lincei Rc. *36*, 113 (1964).

[9] M. BRUFANI, W. FEDELI, G. GIACOMELLO and A. VACIAGO, Experientia *20*, 339 (1964).

[10] J. LEITICH, V. PRELOG and P. SENSI, Experientia *23*, 505 (1967).

[11] The crystal data listed above are based on the interpretation of the electron density peaks not belonging to the molecule of rifamycin as one molecule of dimethylsulphoxide, one molecule of water and one spurious peak per molecule of rifamycin. A different interpretation, which would lead to slightly different values of *M*, D_c, F(000) and μ, is discussed at the end of this paper.

[12] Compare with $D_m = 1.23$ gcm^{-3} for the *p*-iodoanilide of rifamycin B[9].

[13] This number includes F(000).

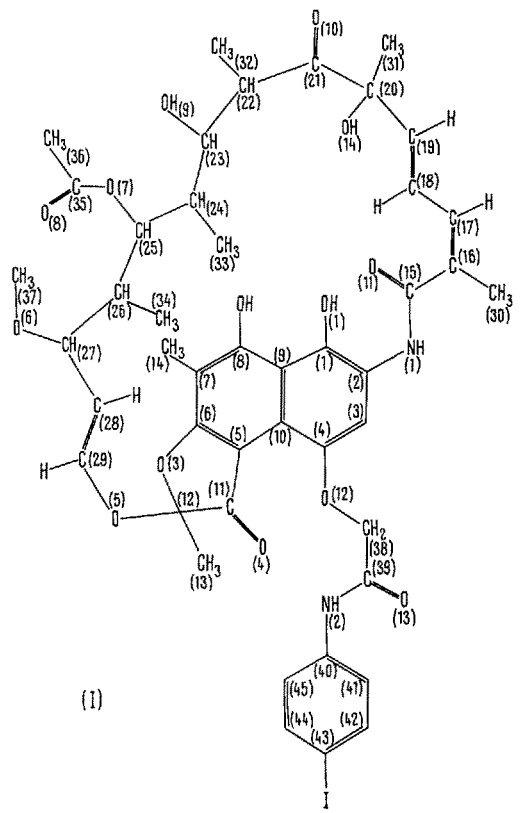
[14] From the Patterson synthesis it was possible to locate also the sulphur atom of the dimethylsulphoxide molecule. However, the pseudo-symmetry would not have been removed by the introduction of the sulphur atom, because the latter was too near to the pseudo-mirror plane (compare Table). The sulphur parameters were therefore not used for phasing the first electron density synthesis. Moreover, it was only later proved beyond doubt, as a result of the structure determination itself, that solvent molecules were present in the crystal structure.

Atomic coordinates

Atom	x/a	y/b	z/c	Atom	x/a	y/b	z/c
C(1)	0.7919	0.2451	0.7037	C(35)	1.5029	0.1525	0.9774
C(2)	0.8497	0.3105	0.7130	C(36)	1.6497	0.1484	0.9482
C(3)	0.9573	0.3206	0.6667	C(37)	1.4610	−0.0293	0.8544
C(4)	1.0639	0.2707	0.6366	C(38)	1.2427	0.3498	0.6050
C(5)	1.0879	0.1389	0.5943	C(39)	1.3574	0.3549	0.5603
C(6)	1.0011	0.0696	0.5917	C(40)	1.5693	0.2904	0.4942
C(7)	0.8839	0.0643	0.6279	C(41)	1.6586	0.3455	0.4793
C(8)	0.7998	0.1165	0.6688	C(42)	1.7802	0.3319	0.4343
C(9)	0.8424	0.1946	0.6696	C(43)	1.8162	0.2673	0.4368
C(10)	0.9879	0.2023	0.6323	C(44)	1.7350	0.2109	0.4625
C(11)	1.2026	0.1266	0.5524	C(45)	1.6215	0.2211	0.5037
C(12)	1.2180	0.0505	0.5281	N(1)	0.7870	0.3662	0.7507
C(13)	1.2191	0.0319	0.4339	N(2)	1.4241	0.2949	0.5369
C(14)	0.7973	−0.0142	0.6468	O(1)	0.6645	0.2271	0.7417
C(15)	0.6535	0.3882	0.7669	O(2)	0.6864	0.1043	0.7005
C(16)	0.6158	0.4396	0.8193	O(3)	1.0802	0.0198	0.5622
C(17)	0.6731	0.4469	0.8975	O(4)	1.3061	0.1636	0.5250
C(18)	0.7620	0.3903	0.9361	O(5)	1.3188	0.0164	0.5697
C(19)	0.8134	0.3959	1.0189	O(6)	1.3459	0.0172	0.8779
C(20)	0.8988	0.3395	1.0839	O(7)	1.4219	0.1761	0.9128
C(21)	1.0656	0.3688	1.0824	O(8)	1.4470	0.1345	1.0364
C(22)	1.1482	0.3537	0.9966	O(9)	1.1417	0.2298	1.0547
C(23)	1.2214	0.2719	0.9971	O(10)	1.0905	0.3930	1.1559
C(24)	1.2146	0.2437	0.9102	O(11)	0.5615	0.3498	0.7299
C(25)	1.2760	0.1674	0.9125	O(12)	1.1864	0.2831	0.6010
C(26)	1.2135	0.1234	0.8291	O(13)	1.4186	0.4152	0.5562
C(27)	1.3259	0.0722	0.8055	O(14)	0.8859	0.2755	1.0213
C(28)	1.2809	0.0317	0.7187	I	2.0218	0.2500	0.3695
C(29)	1.3518	0.0437	0.6520	S	1.3978	0.2596	1.2150
C(30)	0.5340	0.4983	0.7861	C(46)	1.3837	0.3205	1.2963
C(31)	0.8477	0.3241	1.1670	C(47)	1.5264	0.2084	1.2562
C(32)	1.2665	0.4105	0.9935	O(15)	1.2631	0.2215	1.2126
C(33)	1.2722	0.2867	0.8489	O(16)	1.0066	0.4663	0.7835
C(34)	1.0763	0.0880	0.8434				

A second Fourier synthesis, phased on the 'real' set of atoms, enhanced the 'real' image of the molecule. From this point onward the structure was solved completely in several stages, and then refined, by a combination of Fourier methods and block-diagonal least-squares calculations in which atoms were judged by the behaviour of their individual isotropic thermal parameters.

The final structure is summarized in the Table, where the 62 atoms of the molecule (excluding hydrogens) and 5 other atoms, interpreted as 1 molecule of dimethylsulphoxide [S, C(46), C(47), O(15)] and 1 of water [O(16)], are listed. The atomic coordinates of the Table correspond, together with the thermal parameters resulting from the refinement, to a R value of 13.6% and define bond lengths which have an average e.s.d. of 0.07 Å and which deviate by a maximum of 0.24 Å (in one case) and by an average of 0.09 Å from acceptable values for formula (I)[15]. Isotropic thermal motion is assumed for all atoms, except iodine and sulphur, the latter two having been treated anisotropically in the last stages of refinement. Individual isotropic B values are very high, ranging from 4.3 Å² for C(39) to 19.3 Å² for C(36), with an average of 6.7 Å² for the planar part, i.e. everything included between N(1) and C(13), and of 9.6 Å² for the chain from C(15) to O(5), its side chains and substituents, and the solvent molecules. The overall average is 8.3 Å². These



(I)

[15] The molecule of dimethylsulphoxide is not included in this discussion.

high values are not altogether unusual in the case of natural organic compounds (see for instance the structure of the *p*-bromobenzoate of glaucarubin[16]) and may be related to rapid deterioration of the crystals in the X-ray beam, or to considerable freedom of movement, or to some positional disorder. The e.s.d. on the electron density values of the final Fourier synthesis is 0.22 eÅ^{-3}.

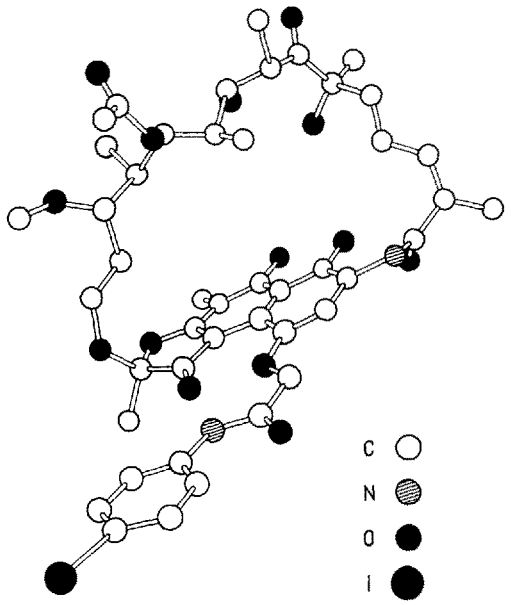


Fig. 1. Stereo-model of the structure of the *p*-iodoanilide of rifamycin Y from X-ray analysis.

All the available crystallographic evidence was taken into account in order to establish structure (I) unambiguously, including bond lengths, electron population of the Fourier synthesis maxima, and stereochemical considerations such as the approximate coplanarity of atoms C(20), C(21), C(22), O(10) or the hydrogen bond distance of 2.63 Å between O(9) and O(14). A stereo-model of formula (I) is given in Figure 1, showing the configuration of the asymmetric carbon atoms and many other configurational and conformational details. The electron density distribution from a Fourier synthesis phased on the positions of the Table is shown by means of superimposed contour sections in Figure 2. Figure 3 gives an idea of the mutual arrangement of the molecules of the *p*-iodoanilide of rifamycin Y, of dimethylsulphoxide and of water in the unit cell.

The structure of rifamycin Y is very similar to that of rifamycin B. The only important differences are illustrated in Figure 4.

The oxygen atom O(10) at C(21), which is a hydroxyl oxygen in rifamycin B, is a carbonyl oxygen in rifamycin Y, and the hydrogen atom at C(20) in rifamycin B is substituted by the hydroxyl oxygen O(14) in rifamycin Y.

The carbon atom C(20) in rifamycin Y has the 20*R* configuration, i.e. the hydrogen on that atom in rifamycin B is replaced by a hydroxyl in rifamycin Y with retention of the configuration. The relative, and therefore the absolute[17], configurations of all the other chirality centres are the same in both compounds. The configurations of the double bonds are also the same.

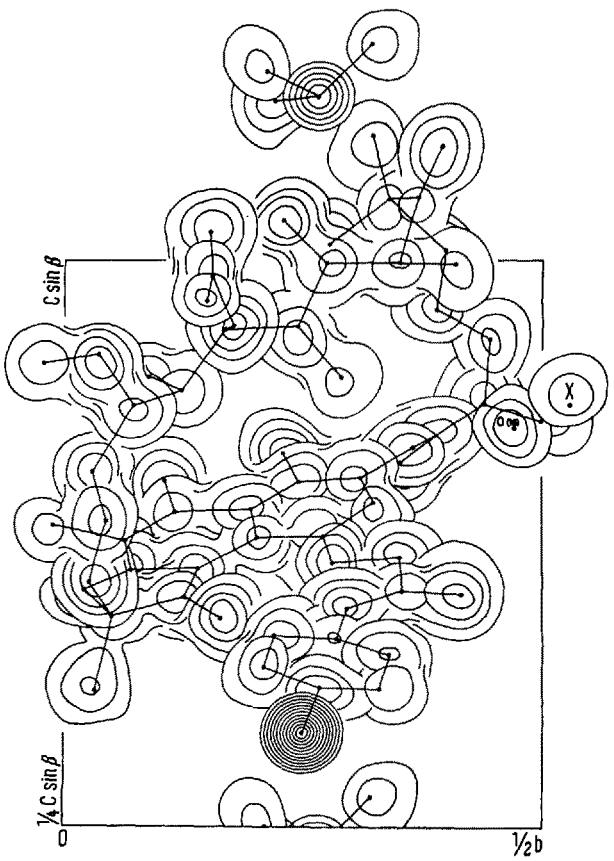


Fig. 2. The final three-dimensional electron density distribution shown by means of superimposed contour sections projected down the *a* axis.

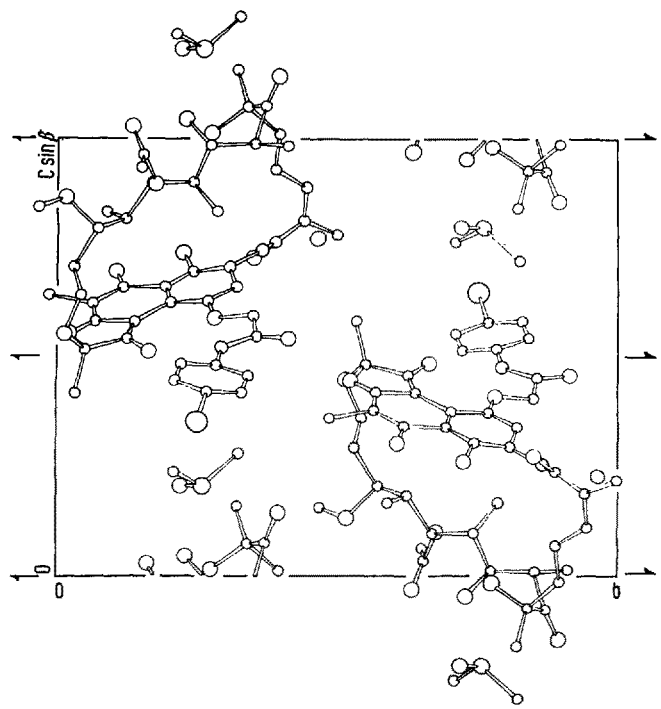


Fig. 3. Packing of the structure seen down the *a* axis. Space group $P2_1$.

[16] G. KARTHA and D. J. HAAS, J. Am. chem. Soc. *86*, 3630 (1964).
[17] The absolute configuration of rifamycin B is known[6].

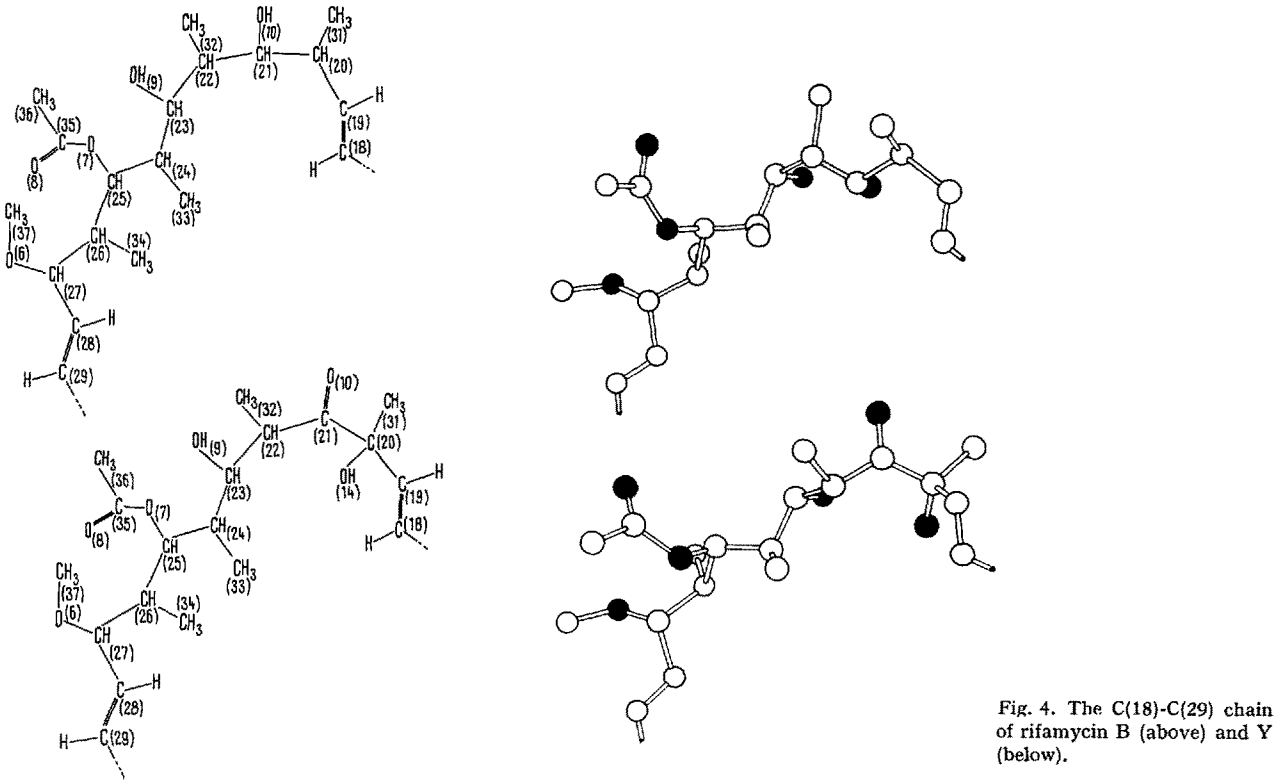


Fig. 4. The C(18)-C(29) chain of rifamycin B (above) and Y (below).

The conformation is practically the same everywhere, except for the (20), (21), (22) portion of the aliphatic chain, where the conformation is different owing to the changed constitution at positions (20) and (21). As already mentioned, O(9) and O(14) are at the hydrogen bond distance of 2.63 Å. In the ester group at C(25) the oxygen atom O(8) is *cis* to the alcoholic carbon C(25): this is known to be the preferred conformation of the ester group in relation to saturated ring systems[18].

There are 2 short, possibly hydrogen bonded, intermolecular contacts between the main molecule and the molecules of dimethylsulphoxide and water: O(15) is at 2.70 Å from oxygen O(9), and O(16) at 2.87 Å from nitrogen N(1).

A brief comment about the solvent molecules present in the crystal structure is necessary. The peak indicated by X in the electron density distribution of Figure 2 has so far been considered as spurious. The presence of spurious peaks predominantly due to series imperfections even in the final Fourier syntheses is not altogether unexpected in the case of large molecules and poor data. However, the presence of the electron density blob X at (1.0334, 0.5325, 0.8128), position (48), i.e. at a distance of 1.36 Å from O(16), and with a population in the same region of that of other maxima interpreted as carbon atoms, gave rise to a stimulating problem which still remains open. This maximum could not be interpreted as either a second water molecule, because of its 'bond' distance from O(16), nor as an alternative position for a statistical distribution of 1 water molecule on positions O(16) and X(48), because the total electron population of O(16) and X(48), even if only approximately calculable, is well over 10 and because O(16) should be stabilized by the hydrogen bond to N(1). The interpretation of X(48) as a spurious peak was therefore adopted at this point. There was however a second interpretation: the addition of a carbon atom with a B value of 10 Å^2 in position X(48) would lower the R value to 12.9% and the spatial and thermal parameters of the structure would then further refine, in 1 cycle, to $R = 12.7\%$. The maxima O(16) and X(48) could therefore be interpreted as 1 molecule of methanol. We have found, using gas chromatography, that methanol is indeed present in the mother liquor when the *p*-iodoanilide of rifamycin Y crystallizes from water-dimethylsulphoxide solutions, i.e. when small quantities of hydroiodic acid are formed due to decomposition of the *p*-iodoanilide derivative. We have also found that methanol is generally formed in solutions of dimethylsulphoxide and hydroiodic acid. But, up to now, we have failed to prove the presence of methanol in crystals of the *p*-iodoanilide of rifamycin Y by NMR. This remains an open question, since the large sample used for the NMR measurements on a 60 MHz Varian instrument (about 25 mg) was made up of small crystals that might well have been different from those used for the crystal structure determination. However, for the time being we favour the safer interpretation of the X maximum as a spurious peak. It should be noted that the new set of parameters at $R = 12.7\%$ and the set at $R = 13.6\%$, given and discussed in this paper, are practically identical as far as the molecules of the *p*-iodoanilide of rifamycin Y and of dimethylsulphoxide are concerned.

[18] A. McL. Mathieson, Tetrahedron Lett. No. 46, 4137 (1965). We take this opportunity to modify a conformational detail of the structure of rifamycin B as published in 1964: the 'labels' C(36) and O(8) should be interchanged in the Table of atomic coordinates and in the Figures 2, 3 and 4 of our 1964 paper in Experientia[9], thus obtaining the above mentioned preferred conformation of the ester group also in the case of rifamycin B. This is the only important change in the structure of the *p*-iodoanilide derivative of rifamycin B found during the final refinement now completed and to be published elsewhere.

Riassunto. La struttura cristallina della *p*-iodio anilide della rifamicina Y è stata determinata con metodi tridimensionali a temperatura ambiente. La cella elementare monoclina, gruppo spaziale $P2_1$, ha i parametri $a = 9{,}49$, $b = 18{,}96$, $c = 15{,}30$ Å, $\beta = 90°48'$, e contiene due molecole.

La costituzione e la configurazione della rifamicina Y, $C_{39}H_{47}NO_{15}$, risultano quindi determinate attraverso lo studio cristallografico della sua *p*-iodio anilide. Anche molte informazioni sulla configurazione della rifamicina Y possono essere tratte dalla configurazione allo stato solido di questo suo derivato.

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[19] We are grateful to Professor P. SENSI of Lepetit SpA Research Laboratories, Milan, for making available supplies of the *p*-iodoanilide of rifamycin Y. We also thank Mr. N. OCELLO for his skillful technical assistance. All the calculations were carried out on the Rome University IBM 7040 computer.

Studies on the Possibility of Epoxide Formation from Disubstituted Hexitols

It was demonstrated by ELSON[1] that in the hematological effects of 1,6-dimethanesulphonyl-D-mannitol (Ia, dimesylmannitol, DMM) a myelotoxicity of the myleran type was combined with some features reminiscent of the lymphotoxic effects of epoxides. On this basis he suggested that in vivo DMM may be converted to epoxide intermediates by splitting off methanesulphonic acid. DAVIS and ROSS[2] investigated the hydrolysis of DMM at pH 7.5, and demonstrated the production of epoxide by addition of thiosulphate to the reaction mixture and acidic titration of the alkalinity liberated in the course of the Bunte-salt formation; the scheme is shown in (Ia–III).

With regard to the chemical analogy between dimesylmannitol and 1,6-dihalogeno-hexitols we were greatly interested in what role may be attributed to an epoxide intermediate in the cytostatic action of dibromomannitol (Ib, DBM, Myelobromol®). We applied to DBM the procedure described by DAVIS and ROSS, and we compared the behaviour of DBM with that of DMM and dibromoerythritol (V, DBE).

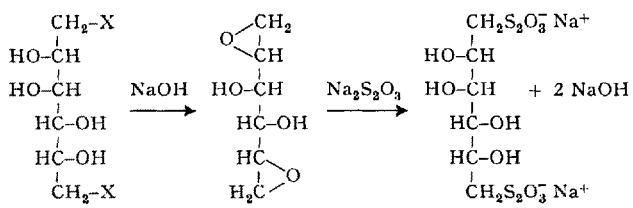
A mixture of 1 m*M* of disubstituted polyol and 10 ml of water was kept at 37 °C and the pH was maintained at 7.5 by continuous addition of 0.1 *N* NaOH as long as the reaction mixture still consumed hydroxide. With DMM and DBM the hydrolysis took 5–6 h, with DBE only $^1/_2$–1 h. To an aliquot part 3–5 g of $Na_2S_2O_3$/m*M* of substance was added, and the alkali set free titrated with 0.1 *N* HCl. The amount of acid required was a measure of the epoxide formed. The calculated epoxide content in the case of DMM, DBM and DBE was 70–75%, 50–55% and 50%, respectively.

These results were inconsistent with the assumed biological role of an epoxide intermediate since the cytostatic activity of DBM is confined selectively to the inhibition of the myeloid system[3,4] in contrast with the lymphotoxic character of the epoxides.

Our attempts to isolate the diepoxide of mannitol, or any other identifiable product, from the reaction mixtures were unsuccessful, and it seemed to us somewhat improbable that 1,6-disubstituted hexitols should yield a product containing energetically unfavourable 3-membered epoxide rings although the presence of 4 hydroxyl groups could give rise to the formation of non-strained 5- or 6-membered internal anhydrides.

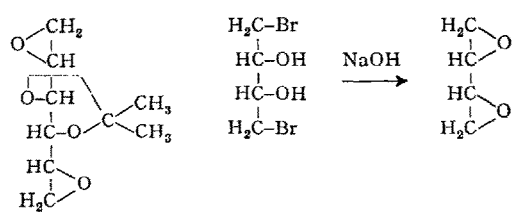
To settle the question, hydrolysis of DMM was carried out in heavy water with NaOD, and NMR measurements were performed on the reaction mixture immediately after the alkaline treatment.

The identification of epoxide rings by proton resonance spectroscopy is based on the observation[5] that epoxide



(Ia) X = OSO_2CH_3 (II) Diepoxide (III) Bunte's salt
(Ib) X = Br

Tentative reaction scheme for the hydrolysis of 1,6-disubstituted hexitols.



(IV) (V) (VI)

1,2-5,6-Dianhydro-3,4-isopropylidene-D-mannitol — 1,4-Dibromo-1,4-dideoxy-meso-erythritol — Meso-1,2-3,4-diepoxybutane

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[3] L. INSTITÓRIS, I. P. HORVÁTH and E. CSÁNYI, Int. Symp. Chemother. *III*, 250 (1963).
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[5] R. A. Y. JONES, A. E. KATRITZKY, J. N. MURRELL and N. SHEPPARD, J. chem. Soc. 2576 (1962).