X-Ray Structure of the Antibiotic Rutamycin

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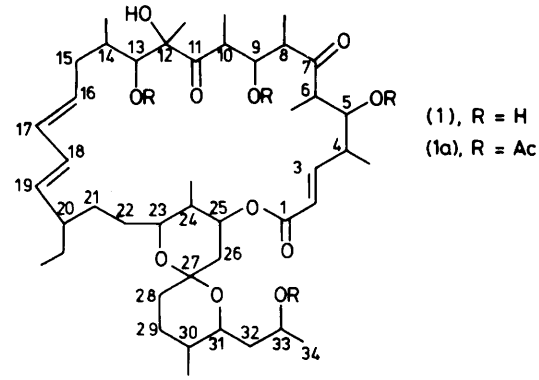
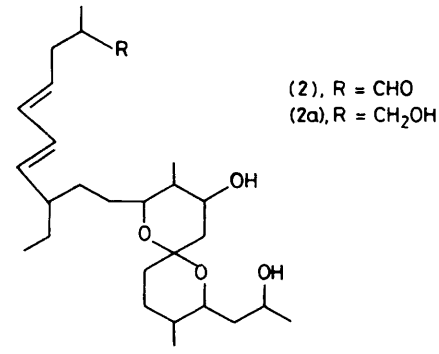
Summary The 26-membered macrolide structure (**1**) has been established for the antibiotic rutamycin by *X*-ray crystallography.

FROM the culture medium of a strain of *Streptomyces griseus* (strain SAB 5843) we have isolated a compound possessing properties resembling those of rutamycin,[1] an antifungal antibiotic of hitherto unknown structure, first obtained[2] from a strain of *S. rutgersensis*. The identity of this newly isolated compound was established by a comparison of its i.r., u.v., ^{1}H n.m.r., and mass spectra and t.l.c. with those of an authentic specimen† of rutamycin. We report here the 26-membered macrolide structure (**1**) for rutamycin.

Silica gel column chromatography of the crude extract from the *S. griseus* culture medium furnished fractions [chloroform–n-hexane–methanol (75:23:2) as eluent], homogeneous on t.l.c., which after crystallisation from n-hexane–diethyl ether deposited crystals of (**1**), $[\alpha]_D^{20}$ −62° (*c* 1·36, $CHCl_3$), m.p. 116—119 °C. An unequivocal molecular weight determination could not be achieved from the mass spectrum of rutamycin itself.[3] However, the electron impact mass spectrum of its tetra-acetate[3] (**1a**) exhibited the molecular ion peak at *m/e* 944 (shifted to *m/e* 956 for the deuterioacetylated derivative) while the chemical ionisation (using isobutane as the reagent gas) mass spectrum displayed the MH^+ ion peak at *m/e* 945. High resolution mass spectrometry provided a molecular formula $C_{52}H_{80}O_{15}$ for the tetra-acetate from which the molecular formula $C_{44}H_{72}O_{11}$ (*M* 776) could be deduced for rutamycin.

U.v. and mass spectral studies established[3] the structural relationship of rutamycin to the oligomycin group of antibiotics. The presence of three double bonds in rutamycin was indicated by the conversion (H_2; 10% Pd–C in EtOH) of (**1a**) into its hexahydro-derivative ($M^{+\cdot}$ 950). A number of features in the ^{1}H n.m.r. spectrum (250 MHz; $CDCl_3$) of rutamycin tetra-acetate could be assigned to structural facets of (**1a**). The conjugated *trans* diene system[4] was observed as two quartets centred at δ 6·0 [C(17)-H, C(18)-H, $J_{16,17} = J_{18,19} = 15$, $J_{17,18}$ 10 Hz] and a complex pattern between δ 5·3 and 5·6 [C(16)-H and C(19)-H]. The *trans* olefinic function of the conjugated lactone system appeared at δ 5·86 [C(2)-H, d, $J_{2,3}$ 15 Hz] and 6·84 [C(3)-H, q, $J_{3,4}$ 9 Hz]. The latter is coupled to the methine proton C(4)-H at δ 2·60 (m) as demonstrated by spin decoupling studies. In addition to the four acetyl methyl groups, δ 2·1 (12H, br s), the ^{1}H n.m.r. spectrum of (**1a**) also displayed methyl signals between δ 0·75 and 1·3 intergrating for *ca.* 30 protons. Signals at δ 1·3 (3H, s), 1·25 (3H, d, *J* 6 Hz), and 0·82 (3H, t, *J* 7 Hz) are assigned



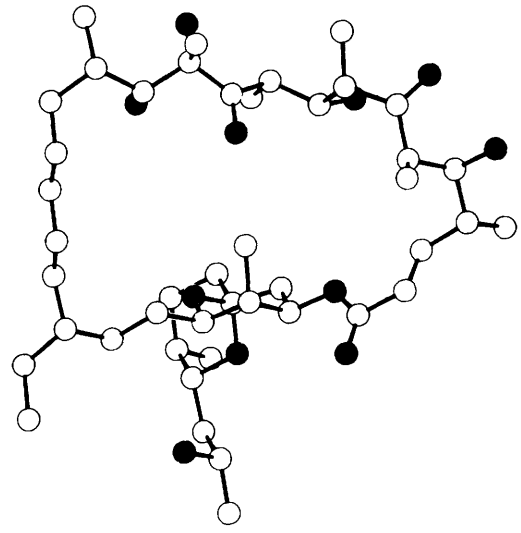


† Obtained through the kind courtesy of Lilly Research Laboratories, Indianapolis, Indiana 46206.

to the methyl group at C(12), the terminal C(34) methyl group, and the ethyl chain at C(20), respectively. The remaining secondary methyl groups appeared as closely packed doublets. Noise- and single frequency-decoupled ^{13}C n.m.r. spectra (22·63 MHz; $CDCl_3$; rel to Me_4Si) of rutamycin displayed in the low-field region the presence of six olefinic methine carbons (δ 122·64, 129·85, 130·76, 132·57, 137·45, and 148·89 p.p.m.), one lactone carbonyl carbon (δ 165·14 p.p.m.), two ketone carbonyl carbons (δ 219·86 and 220·77 p.p.m.), and two tetrasubstituted carbon atoms [δ 83·12 {C(12)} and 97·36 {C(27)} p.p.m.].

Base hydrolysis[3] of rutamycin provided an aldehyde (^1H n.m.r.: δ 9·67; $M^{+\cdot}$ 450, $C_{27}H_{46}O_5$) with concomitant collapse of the lactone ring and *retro*-aldol cleavage between C(12) and C(13). The aldehyde (**2**), which could be reduced by $NaBH_4$ to a dihydro-derivative (**2a**, $M^{+\cdot}$ 452; triacetate $M^{+\cdot}$ 578), retained the conjugated diene system [^1H n.m.r.: δ 5·95 (2H, 2 × q), and 5·2—5·6 (2H, complex m); ^{13}C n.m.r. : δ 127·38, 129·79, 133·23, and 137·91] and a tetrahydro-substituted carbon atom (^{13}C n.m.r. δ 97·42). These data, along with a consideration of the molecular formula of (**2**), suggested its bicyclic structure. The possibility that the two ring systems in (**2**) (consequently, in rutamycin) could be joined through a spiro carbon linked to two oxygen atoms was intimated by the ^{13}C chemical shift (δ 97·42 p.p.m.) of the tetrasubstituted carbon atom as well as the structural relationship of rutamycin to oligomycin B.[5]

The final structure assignment for rutamycin rests on its single-crystal *X*-ray diffraction analysis in combination with the information available from the above spectral data. The crystals of rutamycin are monoclinic, space group $P2_1$, $a = 20·090(3)$, $b = 11·102(3)$, $c = 11·211(5)$ Å $\beta = 102·75(3)°$; $Z = 2$. Intensity data were measured with a Philips PW1100 diffractometer using the ω–2θ scan technique with graphite-monochromated Cu-K_α radiation. Owing to the small size of the crystal (0·1 × 0·1 × 0·4 mm) only 2828 reflexions were collected. We tried to solve the structure by direct methods (MULTAN)[6] but the solution could not be refined and seemed to be displaced in the lattice. From the conclusions derived from the spectral data about the similarities between rutamycin and oligomycin B, the structure of which was determined by Norrestam,[5] we used a Patterson search program[7] with the most characteristic part of the molecule, namely, the two spiro-linked six-membered rings, both in the chair conformation. Full-matrix least-square refinement with isotropic temperature factors resulted in an *R*-factor of 0·098 for 1921 observed reflexions. A three-dimensional view of the



FIGURE

molecule is shown in the Figure. Rutamycin crystallizes with 3 molecules of water which form strong intermolecular hydrogen bonds with the oxygen atoms of the molecule.‡

The structures of rutamycin and oligomycin B are closely similar. The difference lies in the absence of a methyl group on C(26) and the lack of the ketonic function at C(28) in rutamycin (**1**).

We are indebted to Professor Norrestam for supplying the co-ordinates of oligomycin B for comparison.

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‡ The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

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