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Structure of Halomicin B

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Summary The antibiotic halomicin B has been shown by spectroscopic and chemical means to have the structure (1).

HALOMICINS¹ A—D are a new group of antibiotics produced by *Micromonospora halophytica*, and are highly active against gram positive bacteria. We report here the structure of halomicin B.

Halomicin B† (1) crystallises (benzene solvate) as bright yellow needles, $C_{43}H_{58}N_2O_{12}$, m.p. 178—182°, $[\alpha]_D + 73\cdot1°$, $\lambda_{max} 238$ (ϵ 42,161), 298 (14,187), and 415 nm (20,341), $\nu_{max} 3448$, 3390, 1724 (ester), and 1667 (NHCO and aromatic CO) cm⁻¹. As halomicin B is paramagnetic, it was not possible to obtain a good n.m.r. spectrum. On methylation with diazomethane halomicin B gave a mixture of products; column chromatography gave pure halomicin B di-O-methyl ether (2); $C_{45}H_{62}N_2O_{12}$; $(M + 1)^+$, m/e823·44050 (calc. 823·43809); m.p. 156—158°; $[\alpha]_D +$ 313·6°; δ —0·61, 0·65, 0·88, 1·04, and 1·25 (each d, J 7 Hz, Me), 1·82 (OAc), 2·09 (s, =CHMe), 2·38 (overlapping signals from 12-Me and ArMe), 3·02 (aliphatic OMe), 3·76 and 3·90 (each ArOMe), 4·92 [overlapping signals from 28-H (dd, J12·5 and 4 Hz) and 25-H (dd, J 10 and 0·5 Hz]], 5·96 (dd, J 12·5 and 1 Hz, 29-H), 6·12 br (dd, J 15·5 and 6 Hz, 19-H),



(1) $R^1 = R^2 = R^3 = R^4 = H$, X = O(2) $R^1 = Me$, $R^2 = R^3 = R^4 = H$, X = O(3) $R^1 = Me$, $R^2 = COMe$, $R^3 = R^4 = H$, X = O(4) $R^1 = Me$, $R^2 = R^3 = COMe$, $R^4 = H$, X = O(5) $R^1 = Me$, $R^2 = R^4 = COMe$, $R^3 = H$, X = O(6) $R^1 = Me$, $R^2 = R^3 = R^4 = COMe$, X = O(7) $R^1 = Me$, $R^2 = R^3 = R^4 = H$, X = H, OH (8) $R^1 = Me$, $R^2 = R^3 = R^4 = COMe$, X = H, OAc

6.22 (dd, J 10 and 1 Hz, 17-H), 6.54 (dd, J 10 and 15.5 Hz, 18-H), 8.18 [exchanges with D_2O , (-CONH-)], and 8.56 (3-H). Decoupling experiments confirmed the mutual coupling of 17-, 18-, and 19-H. The abnormally high-field methyl doublet at $\delta - 0.61$ suggests an ansamycin structure for (2). The mass spectral fragmentation pattern of (2) parallels the fragmentation pattern of rifamycin SV tri-O-methyl ether. Compound (2) on acetylation at room temperature yielded a mixture of (3) and (4) which were separated using preparative t.l.c.: compound (3), $C_{47}H_{64}N_2O_{13}$, m.p. 236–238°, $[\alpha]_D + 265^\circ$; compound (4), $C_{49}H_{66}N_2O_{14}$, m.p. 233–235°, $[\alpha]_D + 134^\circ$.

On heating with acetic anhydride and pyridine compound (3) yielded mainly (5) and (6): compound (5), $C_{49}H_{66}N_2O_{14}$, $[\alpha]_{p}$, +232° and compound (6), $C_{51}H_{68}N_2O_{15}$, $[\alpha]_{D}$ + 210.8°.

Compound (2) on reduction with NaBH₄ yielded a crystalline 11-hydroxy-compound (7), $C_{45}H_{64}N_2O_{12}$, m.p. 156—158°, $[\alpha]_D + 370.7^\circ$ which showed a singlet for 11-H. Acetylation of (7) yielded a tetra-acetyl derivative (8), m.p.





148—150°, $[\alpha]_D + 168\cdot3^\circ$. The n.m.r. and mass spectra of (3)—(8) were consistent with the assigned structures.

From the above observations it became clear that halomicin B and rifamycins have the same structure of the ansa rings and also that in halomicin B a $C_6H_{12}NO$ unit is attached to the aromatic portion of the molecule. The

† All new compounds gave satisfactory analytical data. Unless otherwise specified, i.r. spectra were recorded in CHCl₃ solution, u.v. spectra in MeOH solution, n.m.r. spectra at 100 MHz in CDCl₃ solution, and $[\alpha]_D$ and c.d. data in MeOH solution. Highresolution mass spectra were obtained using the photoplate recording method on a CEC 21-110B spectrometer.

[‡] The details of the mass spectral fragmentation will be discussed elsewhere.

basic nature of the nitrogen atom (in the side chain) was shown by non-aqueous titration of halomicin B di-Omethyl ether (2) (ΔHNP § = 7.6) which compared very



closely with the corresponding value for p-methoxyaniline $(\Delta HNP = 7.1)$. ΔHNP values for other model compounds were: caprolactam (-36.5), cyclohexanone oxime $(-22\cdot3)$. Acetanilide did not titrate as a base under the above conditions.

Halomicin B (1) on treatment with nitrous acid yielded rifamycin S^{2a} (9) (identical with an authentic sample, m.p. $[\alpha]_D$, t.l.c., i.r., u.v., n.m.r., and mass spectra) and a basic compound (10).

Compound (10), $C_{6}H_{13}NO$ (*M*⁺ 115), m.p. 88°, $[\alpha]_{D}$ $+49\cdot3^{\circ}$ is a strong base and forms salts easily. It is highly sensitive to air and undergoes extensive degradation when set aside. The n.m.r. spectrum of (10) shows signals at δ 1·21 (3H, d, J 6 Hz, Me) 1·67—1·95 (m, 2 × CH₂), 2·97— 3.26 (m, NCH₂ and NCH), and 3.88 (1H, octet, J 3 and 6 Hz). Irradiation of the methyl group collapses the octet at δ 3.88 into a doublet (J 3 Hz) and irradiation of (NCH) collapses the same octet to a quartet (1 6 Hz). On acetylation compound (10) yielded a NO-diacetate (11) as an oil, $[\alpha]_{D}$ + 90.2°. The n.m.r. spectrum of (11) was suggestive of a mixture of rotamers and showed δ 1.19 (d, J 7 Hz, Me), δ 2.1 and 2.3 (OAc), and 5.38 (1H, octet, J 3 and 7 Hz) besides methylene absorptions. The mass spectral fragmentations of (10) and (11) were consistent with the assigned structures.

The relative stereochemistry of (10) was proven by the conversion of (10) into (12) as follows. Compound (10) on treatment with t-butyl bromoacetate yielded (13), as an oil (distils at 120° and 0.5 mmHg), $C_{12}H_{23}NO_3$ (M⁺ 229), $[\alpha]_D$ + 39.8°. On refluxing for 2 h with benzene containing a catalytic amount of toluene-p-sulphonic acid (13) was converted into (12), oil (distils at room temp. and 0.5 mm-Hg), $[\theta]_{235} - 29,564$ (CF₃CH₂OH). The n.m.r. spectrum of (12) besides showing the expected features from the pyrrolidine portion of the molecule showed signals at δ 1.37 (d, J 6.5 Hz, Me), 3.28 and 3.59 [each d, J 16 Hz, $NCH_2C(O)$ -] and 4.62 (1H, octet, J 4 and 6.5 Hz). The small $H_{5,6}$ coupling indicates that (a) if (12) exists in conformation (14) then the methyl group at C-5 should be axial and (b) if (12) exists in conformation (15) then the methyl group at C-5 could be either axial or equatorial. It was therefore desirable to prepare (16) and study the coupling of the C-5 protons. L-Prolinol³ on treatment with t-butyl bromoacetate yielded (17), as an oil, $C_{11}H_{21}NO_3$ (M+ 215), $[\alpha]_D - 5 \cdot 6^\circ$. On treatment with benzene and a catalytic amount of toluene-p-sulphonic acid (17) was smoothly converted into (16), as an oil, $C_7H_{11}NO_2$ (M⁺ 141), $[\theta]_{235} + 7541$ (CF₃CH₂OH). In the n.m.r. spectrum of (16) the C-5 protons appeared at δ 4.14 (t, J 10 Hz) and 4.45 (dd, J 4.5 and 10 Hz) confirming that (16) exists in conformation (18) rather than (19). As replacement of a hydrogen in (18) or (19) with a methyl group does not cause any serious interaction it is reasonable to assume that compound (12) exists in conformation (14). The absolute stereochemistry of (10) was determined as follows.

Compound (10) on treatment with pyridine and toluenep-sulphonyl chloride was converted into (20), $C_{20}H_{25}NO_5S_2$ $(M^+ 423)$, m.p. 108–109°, $[\theta]_{230} + 17,000$. Reduction of (20) with LiAlH₄ yielded (21), $C_{13}H_{19}NO_2S$ (*M*⁺ 253), m.p. 56—57°, $[\theta]_{228}$ + 7122. Similarly starting with L-prolinol and following the above sequence of reaction (22) was prepared. Compound (22),³ m.p. 68-69°, C₁₂H₁₇NO₂S (M+ 239) showed a negative Cotton effect in its c.d. $[\theta]_{228} - 5333$ thus establishing the R-configuration for (21). The stereochemistry of (10) is therefore (2R,3S). As it was pointed out earlier that halomicin B on treatment with nitrous acid yielded rifamycin S (9) and the pyrrolidine (10) it was considered desirable to reconstruct halomicin B from a rifamycin derivative. Rifamycin 'O' (23) was heated4 with (10) in tetrahydrofuran and from the reaction mixture we isolated halomicin B which was shown to be identical with the naturally occurring compound (m.p., $[\alpha]_D,$ i.r., u.v., m.s.) thus establishing the structure and absolute stereochemistry of halomicin B.

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§ ΔHNP = Effective basicity of the compounds and is represented by the differences in their half neutralization potentials from that of 8-hydroxyquinoline. The details of this procedure will be published elsewhere by J. McGlotten.

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