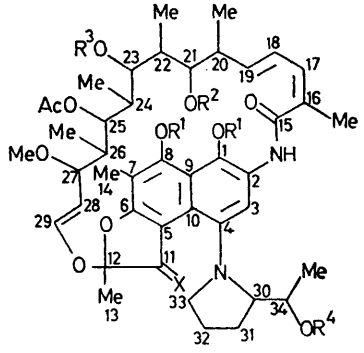
Structure of Halomicin B

By ASHIT K. GANGULY,* SOL SZMULEWICZ, OLGA Z. SARRE, DIANNE GREEVES, JAMES MORTON, and JAMES MCGLOTTEN
(*Chemical Research Department, Schering Corporation, Bloomfield, New Jersey* 07003)

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

HALOMICINS[1] 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°, $[\alpha]_D$ + 73·1°, λ_{max} 238 (ϵ 42,161), 298 (14,187), and 415 nm (20,341), ν_{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/e* 823·44050 (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, =CH*Me*), 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, *J* 12·5 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),



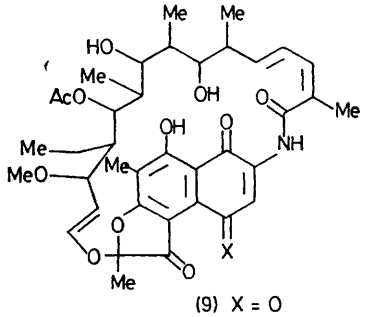
(**1**) $R^1 = R^2 = R^3 = R^4 = H$, X = O
(**2**) R^1 = Me, $R^2 = R^3 = R^4 = H$, X = O
(**3**) R^1 = Me, R^2 = COMe, $R^3 = R^4 = H$, X = O
(**4**) R^1 = Me, $R^2 = R^3$ = COMe, $R^4 = H$, X = O
(**5**) R^1 = Me, $R^2 = R^4$ = COMe, $R^3 = H$, X = O
(**6**) R^1 = Me, $R^2 = R^3 = R^4$ = COMe, X = 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 δ −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.‡

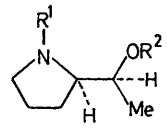
Compound (**2**) on acetylation at room temperature yielded a mixture of (**3**) and (**4**) which were separated using preparative t.l.c.: compound (**3**), $C_{47}H_{64}N_2O_{13}$, m.p. 236—238°, $[\alpha]_D$ + 265°; compound (**4**), $C_{49}H_{66}N_2O_{14}$, m.p. 233—235°, $[\alpha]_D$ + 134°.

On heating with acetic anhydride and pyridine compound (**3**) yielded mainly (**5**) and (**6**): compound (**5**), $C_{49}H_{66}N_2O_{14}$, $[\alpha]_D$, +232° and compound (**6**), $C_{51}H_{68}N_2O_{15}$, $[\alpha]_D$ + 210·8°.

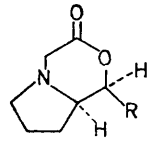
Compound (**2**) on reduction with $NaBH_4$ yielded a crystalline 11-hydroxy-compound (**7**), $C_{45}H_{64}N_2O_{12}$, m.p. 156—158°, $[\alpha]_D$ + 370·7° which showed a singlet for 11-H. Acetylation of (**7**) yielded a tetra-acetyl derivative (**8**), m.p.



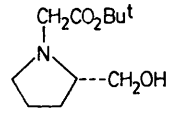
(**9**) X = O
(**23**) X = $-OCH_2C(O)O-$



(**10**) $R^1 = R^2 = H$
(**11**) $R^1 = R^2$ = COMe
(**13**) $R^1 = CH_2CO_2Bu^t$, $R^2 = H$
(**20**) $R^1 = R^2 = p$-Me$C_6H_4SO_2$



(**12**) R = Me
(**16**) R = H



(**17**)

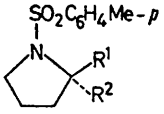
148—150°, $[\alpha]_D$ + 168·3°. The n.m.r. and mass spectra of (**3**)—(**8**) were consistent with the assigned structures.

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

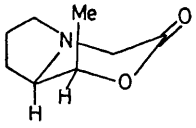
† All new compounds gave satisfactory analytical data. Unless otherwise specified, i.r. spectra were recorded in $CHCl_3$ solution, u.v. spectra in MeOH solution, n.m.r. spectra at 100 MHz in $CDCl_3$ solution, and $[\alpha]_D$ and c.d. data in MeOH solution. High-resolution mass spectra were obtained 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-*O*-methyl ether (**2**) (ΔHNP§ = 7·6) which compared very closely with the corresponding value for *p*-methoxyaniline (ΔHNP = 7·1). ΔHNP values for other model compounds were: caprolactam (−36·5), cyclohexanone oxime (−22·3). Acetanilide did not titrate as a base under the above conditions.



(21) R^1 = Et, R^2 = H
(22) R^1 = H, R^2 = Me



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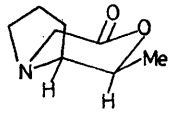

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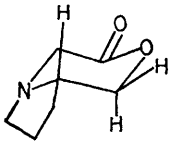

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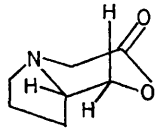

(19)

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**), $C_6H_{13}NO$ (M^+ 115), m.p. 88°, $[\alpha]_D$ +49·3° 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 × CH_2), 2·97—3·26 (m, NCH_2 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 (*J* 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 mmHg), $[\theta]_{235}$ − 29,564 (CF_3CH_2OH). 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-Prolinol[3] on treatment with t-butyl bromoacetate yielded (**17**), as an oil, $C_{11}H_{21}NO_3$ (M^+ 215), $[\alpha]_D$ − 5·6°. 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 (CF_3CH_2OH). 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 toluene-*p*-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 $LiAlH_4$ 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°, $C_{12}H_{17}NO_2S$ (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 (2*R*,3*S*). 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 heated[4] 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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