Communications to the Editor

THE STRUCTURE OF PAULOMYCIN

Sir:

Paulomycin was originally isolated as a mixture of antibiotics[1] now designated paulomycins A and B[2], and its relationship to the senfolomycins[3] and proceomycin[4] was noted. Paulomycins A and B, separated by ARGOUDELIS *et al.*[2], have excellent antibacterial activity against organisms resistant to other antibiotics.

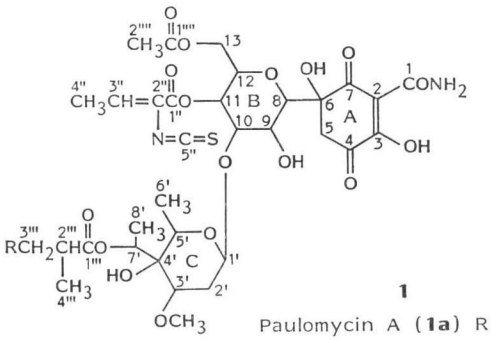
The present communication reports the structures of paulomycins A and B (**1a** and **1b**), which proved to be members of a family of antibiotics of hitherto unreported structures, and the evidence on which these structures are based. The structures depicted do not imply any stereochemistry.

A previous publication[2] has reported the molecular formulas of **1a** and **1b** and established the difference between them as indicated above. The subsequent discussion will be concerned only with paulomycin A unless otherwise indicated. The earlier publication reported physical properties including complete ^{1}H NMR and ^{13}C NMR spectra with complete chemical shifts and multiplicities.

Compound **1a** in solution in CH_3OH is slowly converted to a yellow solid (paulomycinone A, **2**) which was purified by chromatography. Its molecular formula is $C_{34}H_{44}N_2O_{16}S$; mp 57~73°C; $[\alpha]_D$ +2° (*c* 0.694, MeOH), λ_{max}^{MeOH} 232 nm (ε 15,250), 264 (18,400), 440 (2,100); ν_{max}^{Nujol} 3488, 3363, 3255, 3249, 2051, 1735, 1695, 1637, 1618, 1569 cm^{-1}; mass spectrum *m/z* 768. The ^{1}H NMR spectrum of **2** differs significantly from that of **1a** only in that an exchangeable H (δ 5.20 s) signal and the signal from CH_2 alpha to C=O have disappeared with the appearance of a resonance for an olefinic H (δ 6.47 s). In the ^{13}C NMR spectrum the two ketonic carbonyls of **1a** are now represented by characteristic quinone peaks (δ 180.51 s, 185.12 s), and olefinic resonances have appeared at δ 151.96 (s) and 129.59 (d). The molecular formula suggests dehydration of **1a** with formation of a quinone ($Na_2S_2O_4$ decolorization) incorporating the original ketone carbonyls and one olefin. These changes would be consistent with conversion of a moiety **3** to **4**. The moiety **3** would be in accord with IR, ^{1}H NMR and ^{13}C NMR spectra of **1a**.

In a solution of 0.01 N $(CH_3)_3N$, **1a** forms a colorless, crystalline solid (paulomenol A, **5**) which was purified by chromatography and crystallization; mp 187~191°C; λ_{max}^{EtOH}, 241 nm (ε 8,590), 318 (8,860); ν_{max}^{Nujol} 3300, 3270, 1730, 1645, 1580, 1305, 1245 cm^{-1}; mass spectrum [4$(CH_3)_3$Si derivative] *m/z* 949.4221 [Calcd for $C_{29}H_{43}NO_{16}\cdot 4(CH_3)_3Si$: 949.41636]. The change in molecular formula suggests loss of $C_5H_3NO_2S$. The ^{1}H NMR spectrum of **5** no longer has the doublet at δ 1.97 (3H) nor the quadruplet at δ 6.83 for olefinic H. The chemical shift of H-11 at δ 4.82 in **1a** has moved upfield to δ 3.49 in **5**. The argument for H-11 resonating at δ 4.82 in **1a** is the same as that for locating H-11 in ring B of **7**. Five signals have disappeared from the ^{13}C NMR spectrum (δ 14.11, 125.36, 136.64, 142.64, 160.25). The mass spectrum and analysis coupled with the spectral data, which indicate loss of NCS (no 2050 cm^{-1} band in the IR), indicated loss of $CH_3CH{=}C(-C(=O)-)-N{=}C{=}S$. This acyl group (corresponding acid, paulic acid, **6**) was isolated as 5-methylthiazolidin-2-thione-4-carboxylic acid; ^{1}H NMR (CD_3COCD_3) δ 1.57 (1H, d), 4.27 (1H, dq), 4.56 (1H, d); ^{13}C NMR (CD_3COCD_3) δ 198.46 (C=S), 170.90 (C=O), 70.69 (CHN), 47.68 (CHS), 22.45 (CH_3); mass spectrum *m/z* 176.9904 (Calcd for $C_5H_7NO_2S_2$: 176.9918). These data establish attachment of the pauloyl group of **6** to oxygen of C-11 in **1a**.

Acidic methanolysis of **1a** forms an orange, crystalline solid [11-*O*-pauloylpaulinone, **7**] and a colorless liquid [methyl 7-*O*-(2-methylbutyryl)-paulomycoside, **8**]. Compound **7** was purified by column chromatography and recrystallization; mp 157~160°C; $[\alpha]_D^{25}$ +180° (*c* 0.2835, MeOH); λ_{max}^{MeOH}, 231 nm (ε 15,400), 266 (18,050), 438 (1,700); ν_{max}^{Nujol} 3511, 3436, 3417, 3327, 2050, 1722, 1696, 1682, 1644, 1634, 1617, 1568, 1512 cm^{-1}; mass spectrum *m/z* 454. The ^{1}H NMR spectrum retains chemical shifts for the pauloyl moiety and for the fragment **4**. In addition, a sequence of five CHO resonances is present (δ 5.06 d,



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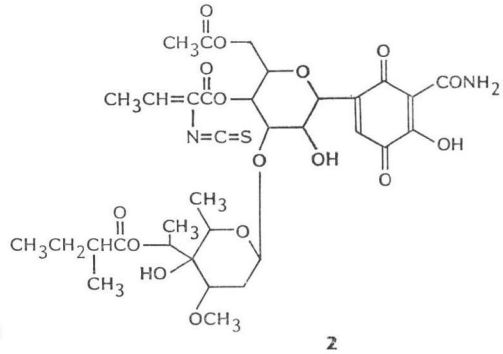
Paulomycin A (**1a**) R = CH_3

Paulomycin B (**1b**) R = H



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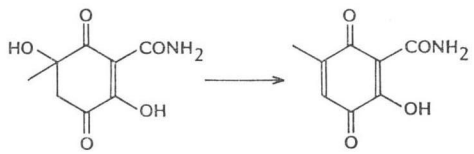
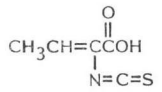


3 **4**



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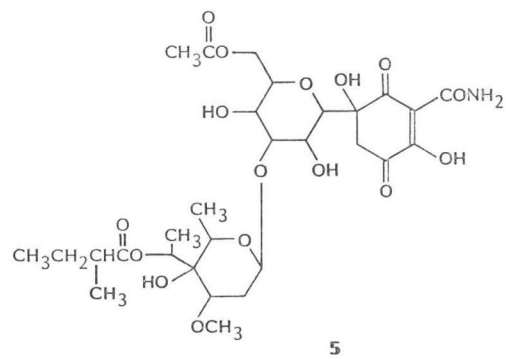

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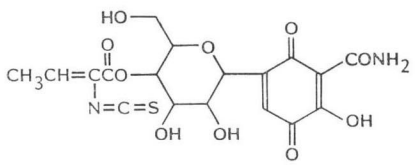

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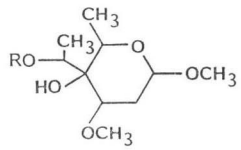
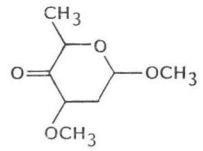


8 R = CH_3CH_2CHCO (with CH_3)

9 R = H



10

3.61 m, 4.45 dd, 4.97 dd, 4.06 m). The signal at δ 5.06, which has moved from 3.83 in **1a**, must arise from H in an allylic position indicating attachment of C-8 to C-6 and the starting point for the above sequence. Coupling constants of $J_{8,9}=10.5$, $J_{9,10}=2$, $J_{10,11}=2.2$ and $J_{11,12}=10$ Hz indicate the sequence and chemical shift values show all of these are on carbon substituted by oxygen. This indicates a tetrahydropyran ring with the δ 4.05 H coupled to CH_2O at δ 3.61. The ^{13}C NMR is also consistent with such a system (δ 78.26 d, 69,70 d, 76.18 d, 70.73 d, 72.29 d, 62.30 t). ^1H NMR chemical shifts at δ 8.56 (1H), 9.93 (1H) and 13.50 (enolic H) substantiate the presence of ring A. The molecular formula of $C_{18}H_{18}N_2O_{10}S$ suggests with the above data that **7** comprises rings A, converted to the moiety **4**, and B with pauloyl attached at C-11. The ^1H NMR signal for H-13 (2H) has moved upfield relative to **1a** from δ 3.94 to 3.61 suggesting loss of acyl from C-13. The acyl must be acetyl as **7** and **8** account for only 32 C of 34 C in **1a** and ^1H NMR and ^{13}C NMR signals in the spectra of **1a** suggest the presence of $CH_3C{=}O$. The position of the acetyl group is borne out by conversion of **7** to its 9,10,13-tri-*O*-acetyl derivative whose H-13 H's resonate at δ 4.27. Since OH has been demonstrated to be present in **1a** at C-9 (coupling to H-9), then **8** must be attached to C-10. The ^1H NMR re-

sonance for H-8 has changed from δ 3.83 in **1a** to 5.06 in **7** indicating attachment of ring B to ring A by a C-8 to C-6 bond and establishing that dehydration must occur at C-5–C-6 necessitating OH at C-6 and CH_2 at C-5 in **1a**.

Compound **8** was purified by chromatography; $[\alpha]_D^{25}-76°$ (*c* 0.722, MeOH); ν_{max}^{neat} 3503, 2970, 2940, 2880, 1734, 1644, 1463, 1382 cm^{-1}; mass spectrum *m/z* 304. Analysis and mass spectra suggest a molecular formula of $C_{15}H_{28}O_6$. **8** was readily hydrolyzed in 0.5 N NaOH in 1:1 MeOH - H_2O to 2-methylbutyric acid, identified by HPLC, NMR and mass spectrum, and a liquid (methyl paulomycoside, **9**) purified by chromatography; $[\alpha]_D^{25}-80°$ (*c* 0.852, MeOH); ν_{max}^{neat} 3479, 2978, 2943, 2907, 2831, 1458, 1421, 1379 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.08 (3H, d, *J*=6.2 Hz), 1.17 (3H, d, *J*=6.2 Hz), 1.95 (1H, m, *J*=3.5, 11.8 Hz), 2.04 (1H, m, *J*=2.5 Hz), 3.23 (3H, s), 3.31 (3H, s), 3.38 (1H, dd, *J*=5, 11.8 Hz), 3.69 (1H, q, *J*=6.2 Hz), 3.87 (1H, q, *J*=6.2 Hz), 4.69 (1H, dd, *J*=2, 3.5 Hz); ^{13}C NMR ($CDCl_3$) δ 98.34 (d), 76.39 (d), 73.92 (s), 71.96 (d), 66.91 (d), 55.22 (q), 54.89 (q), 29.84 (t), 18.93 (q), 13.58 (q); mass spectrum *m/z* 189.1153 (M^+-OCH_3, Calcd for $C_9H_{17}O_4$: 189.1126). The ^{13}C NMR, ^1H NMR, mass spectra and analysis suggest a molecular formula of $C_{10}H_{20}O_5$. The ^1H NMR of **8** has a resonance at δ 5.27 (q) which in **9** is δ 3.69, indicating attachment of the 2-methylbutyryl group at C-7 since only H-5 and H-7 are adjacent to CH_3. Thus **9** must be as indicated below with **8** being the 7-*O*-(2-methylbutyryl) derivative.

Periodate oxidation of **9** forms CH_3CHO, identified as its 2,4-dinitrophenylhydrazone, and a C-8 compound (**10**) which was purified by chromatography; $[\alpha]_D^{25}-274°$ (*c* 0.005, $CHCl_3$); ν_{max}^{neat} 1740 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.30 (3H, d, *J*=6.56 Hz), 2.07 (1H, ddd, *J*=12.91, 3.59, 12.06 Hz), 2.56 (1H, ddd, *J*=12.91, 1.55, 6.77 Hz), 3.46, 3.50 (6H, 2s), 4.23 (1H, dd, *J*=6.77, 12.06 Hz), 4.31 (1H, q, *J*=6.56 Hz), 4.90 (1H, dd, *J*=1.55, 3.59 Hz); ^{13}C NMR ($CDCl_3$) δ 205.42 (C=O), 98.04 (C-1), 78.21 (C-3) 69.99 (C-5), 58.35, 55.40 ($2CH_3O$), 39.41 (CH_2), 13.85 (CH_3); mass spectrum *m/z* 173.0792 [Calcd for $C_8H_{13}O_4$ (M^+–H), 172.0814]. The spectral data are conclusive for structure **10** for this compound. These products substantiate structure **9** for methyl paulomycoside.

The presence of a primary amide in **1a** is indicated by a ^{13}C NMR chemical shift of δ 169.35 and ^1H NMR resonances of δ 8.41 and 9.92 (each 1H) and isolation of NH_3 from base hydrolysis of **5**. This group must be present in **7** as both nitrogen atoms of **1a** remain and in **5** which has only one N. Since this group cannot be present in ring B as indicated by NMR data from **7**, it must be on ring A as shown in **3**. As neither **1a** nor **5** are oxidized by periodate, the A ring ketonic carbonyls must behave as carbonyls of vinylogous acids as seems possible in **3**. If the $CONH_2$ and OH of ring A were interchanged, the C-6 hydroxyl-C-7 carbonyl would necessarily be oxidized by periodate. Consequently, these substituents must be as in **3**. Furthermore, in the ^{13}C NMR of **7**, H-5 is coupled to the enolic C requiring a three-bond connection as in **3**[5].

These data strongly favor structures **1a** and **1b** for paulomycins A and B.

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