ON THE STRUCTURE OF HYGROMYCIN

THE LOCATION OF A METHYLENE SUBSTITUENT AND THE ANOMERIC CONFIGURATION OF THE *ARABINO*-HEXOSIDE MOIETY

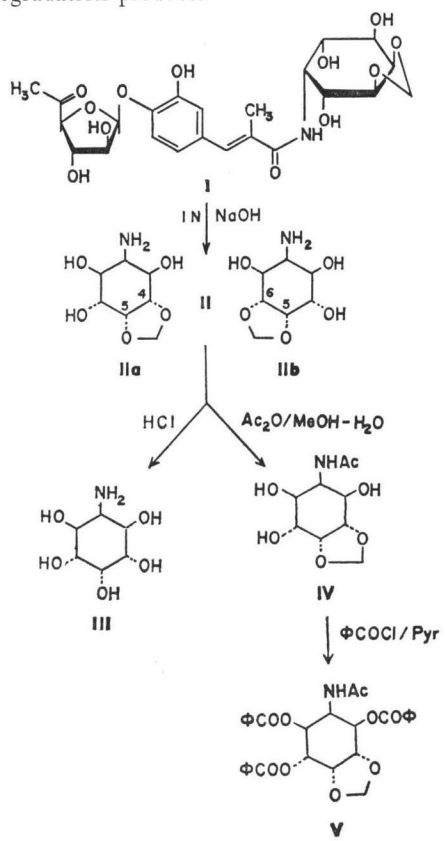
Sir:

Hygromycin was first reported in 1953[1,2] and several identical antibiotics such as homomycin and 1703-18B have been described.[3,4,5] Although the structure of the antibiotic was determined by chemical degradations,[6,7,8] some structural aspects must still be resolved. First, neoinosamine-2 is known to be a constituent with a methylene substitution, whose location, however, has not been definitely established so far. It has been suggested that the substituent is located either on the 4, 5- or the 5, 6-position by the fact that one mole of periodate was consumed by the N-acetate of methylene neoinosamine-2.[9] Second, the anomeric configuration of the 6-deoxy-5-oxo-*arabino*-hexose moiety is also unknown. To solve these problems the following experiments were undertaken employing the antibiotic St-4331 recently isolated in these laboratories from a strain of *Streptomyces* during the systematic screening using *Mycoplasma gallisepticum* as a test organism.

St-4331 (**I**), $C_{23}H_{29}NO_{12}$,[10] mp 113~115°C, $\lambda_{max}^{H_2O}$ 215 nm (log ε 4.28), 272 nm (log ε 4.17), 302 nm (sh, log ε 3.98), was distinctly identified with homomycin by their ir spectra, color reactions and paper chromatographic behavior.[5] Pmr and cmr spectra of **I** were consistent with the proposed structure of hygromycin.[11]

It was previously reported that alkaline hydrolysis of homomycin gave methylene neoinosamine-2 which was not fully characterized as such.[9] Similar procedures were employed to obtain the compound with some modifications. **I** was hydrolysed by 1 N NaOH in a boiling water bath for 1 hour and after cooling, the reaction mixture was adsorbed on Amberlite IR-120 (H^+ form) which was washed successively with distilled water and 5% aqueous pyridine solution. Elution with 1% NH_4OH followed by evaporation provided the methylene neoinosamine-2 (**II**), which in turn was crystallized from methanol-water to yield colorless rods, $C_7H_{13}NO_5$,[10] mp 155~159°C (dec.). Compound **II** was reported to be optically inactive.[9] However, as can be recognized from the structure (**IIa** or **IIb**), the compound should have optical activity because of its dissymmetry, and indeed, it is optically active, $[\alpha]_{574}^{22}$ −33.0° (*c* 1.9, H_2O). Acid hydrolysis of **II** gave optically inactive neoinosamine-2 (**III**), $C_6H_{13}NO_5$,[10] mp 235~239°C (dec.), which was identified with an authentic specimen by mp, mixed mp, and ir spectra.

Fig. 1. Structure of hygromycin (St-4331) and its degradation products



Treatment of **II** with acetic anhydride in methanol-water followed by Sephadex LH-20 column chromatography provided the amorphous N-acetate (**IV**), $C_9H_{15}NO_6$. It has been known that the absolute configuration of α-glycol system can be determined by the modified cuprammonium method[12] and the CD spectrum of **IV** in Cupra A solution has a maximum at 580 nm ([θ]+170) to show δ conformation of the α-diol system in **IV**, as

in the case of methyl 4, 6-O-benzylidene-α-D-glucopyranoside, $[\theta]_{580}^{Cupra\ A}$ +250. Therefore, the diol system must locate on C-6 and C-1 rather than on C-3 and C-4, hence the methylene group was determined to be at the C-4 and C-5 position.

Further support was obtained by benzoylation of **IV** with benzoyl chloride in pyridine at room temperature to give O-tribenzoate (**V**) and N, O-tetrabenzoate (**VI**), $C_{37}H_{31}NO_{10}$ (M^+: *m/e* 649, M−122: *m/e* 527). Compound **V** was crystallized to colorless needles, $C_{30}H_{27}NO_9$,[10] mp 235~238°C, λ_{max}^{MeOH} 230 nm (log ε 4.60), 274 nm (log ε 3.46), 282 nm (log ε 3.37), ν_{max}^{nujol} 3300, 1730, 1720, 1640, 1580, 1280, 1100, 720 cm^{-1}, and mass spectra (M^+; *m/e* 545, M−122: *m/e* 423). The conformation of **V** in methanol was determined to be a twisted boat form by the pmr analysis, δ^{TMS} 5.95 dd (H-1), 5.25 dd (H-2), 5.32 t (H-3), 4.67 br.d (H-4), 4.60 br.d (H-5), 6.13 dd (H-6) and 4.97 s, 5.44 s (-OCH_2O-); $J_{1,2}$ 5.0 Hz, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 3.0 Hz, $J_{4,5}$ 7.0 Hz, $J_{5,6}$ 2.5 Hz, $J_{6,1}$ 8.0 Hz.

If the methylene group of **II** is located on the 4, 5-position as in structure **IIa**, positive induced CDs can be expected of **V** from the exciton chirality rule for the 1, 6-dibenzoyl and possibly for the 1, 3-dibenzoyl systems.[13] On the other hand, an enantiomeric 5, 6-methylene substitution (structure **IIb**) may give rise to the counterclockwise projections of benzoyl groups to show negative COTTON effects. Since the positive CD max, $[\theta]$−43,900, at 237 nm and min, $[\theta]$−12,200, at 221 nm were observed for **V** in methanol, **II** was ultimately concluded to be 1L-4, 5-O-methylene-2-amino-2-deoxy-*neo*-inositol as shown in **IIa**.

Since the anomeric configuration of the antibiotic was not assigned, the cmr spectra of **I** were studied. It has been firmly established that the chemical shifts of individual carbon atoms are exquisitely sensitive to steric crowding, especially by vicinal oxygen substituents and, recent studies on nucleosides,[14] O-glycosides[15] and C-glycosides[16] proved the usefulness of cmr and generalized that the anomeric carbon signal of an isomer having a *cis*-relationship between the aglycon and its C_2-hydroxyl group in furanosides appears at several ppm higher field. C-1, C-2 and C-3 of the *arabino*-hexoside moiety of **I** were observed at 103.5 ppm, 77.5 ppm and 78.0 ppm, respectively,[11] which were almost identical with those of *cis* methyl β-L-arabinofuranoside appearing at 103.3 ppm, 76.3 ppm and 77.9 ppm, respectively. On the other hand, the *trans* compound, methyl α-L-arabinofuranoside were reported to show those signals at 109.5 ppm, 82.0 ppm and 77.9 ppm, respectively.[15] Furthermore, phenyl β-D-ribofuranoside of a model compound with *trans* configuration showed its anomeric carbon at 106.3 ppm,[14] which is 2.8 ppm downfield from that of **I**. Therefore, the anomeric structure of **I** is now assigned to be β-*cis* configuration.

This assignment was further supported by the pmr studies. Although it is not conclusive, it has been reported that the coupling constants of the C-1 and C-2 protons of aldofuranosides are so characteristic that relative configurations at these centers may be assigned.[17] The anomeric proton of **I** was observed at 5.62 ppm (d, J=4.0 Hz) in methanol-d_4 and at 5.92 ppm (d, J=4.0 Hz) in pyridine-d_5, and the acetate showed the anomeric signal at 5.95 ppm (d, J=4.4 Hz) in $CDCl_3$. Their coupling constants support *cis* stereochemistry on the C-1 and C-2 of the *arabino*-hexoside since it is known that J values of 3~5 Hz are expected for *cis*-furanoside configurations and 0~2 Hz for *trans*.[18]

Based on the foregoing data together with the previous results, the structure of hygromycin (homomycin, 1703-18B, St-4331) is determined as **I** in Fig. 1.

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