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# THE CHEMISTRY OF THE LEUCOMYCINS. II.

# STRUCTURE AND STEREOCHEMISTRY OF LEUCOMYCIN $A_3$.

Satoshi Ōmura*, Haruo Ogura** and Toju Hata*,**

Kitasato Institute* and Kitasato University**

Minato-ku, Tokyo, Japan

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In the previous communication[1], it was described that leucomycin $A_3$ is a macrolide containing mycaminose and isovaleryl mycarose. In this paper we propose the full structure of leucomycin $A_3$ (I) and also the stereochemistry of the mycarosidic linkages of spiramycin and magnamycin[2] in relation to leucomycin $A_3$. Dehydroleucomycin $A_3$[1] obtained from I by oxidation with $MnO_2$ is identical with magnamycin B[3] as compared by IR, UV and NMR spectra, and behavior on thin layer chromatography. From these experiments, the structure of I becomes clear. Further support for the structure of I follows from the following experiments.

The pka' values of I, diacetyl leucomycin $A_3$, demycarosyl leucomycin $A_3$, and triacetyl demycarosyl leucomycin $A_3$, 6.70, 5.69, 7.80 and 5.35, respectively[1], suggest that mycarose is attached to either C-2' or C-4' of the mycaminose, i.e., a position neighbouring the dimethylamino group[4]. The position of the mycarose residue can be fixed at C-4' since treatment of O,O-dimethyl leucomycin $A_3$ methyliodide (II) with conc. HCl affords III, which when cleaved by sodium periodate provides acetaldehyde in good yield.

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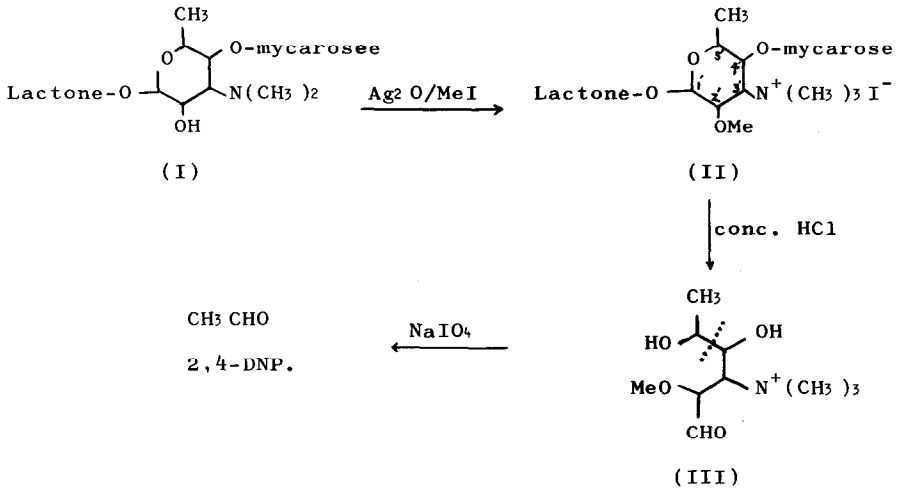
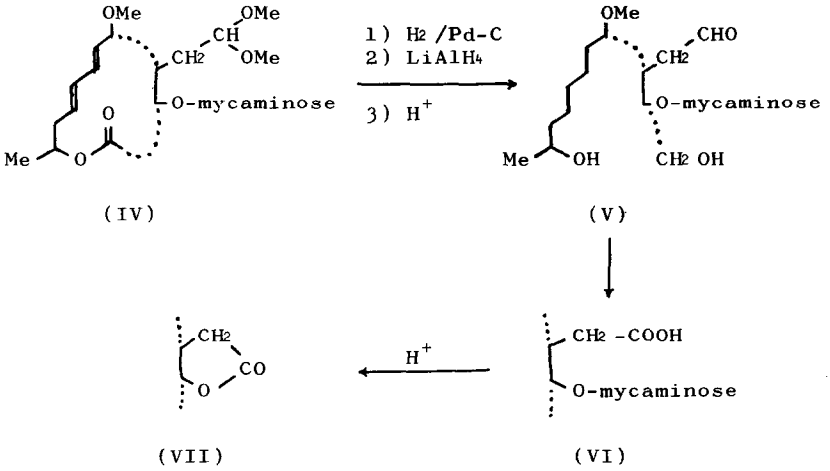
Rotations were measured in chloroform solution at 25°C, UV spectra were taken in methanol solution and pka' values were measured in 50% ethanol. Satisfactory analyses were obtained for all compounds for which molecular formulae are given.




(I) (II) (III)

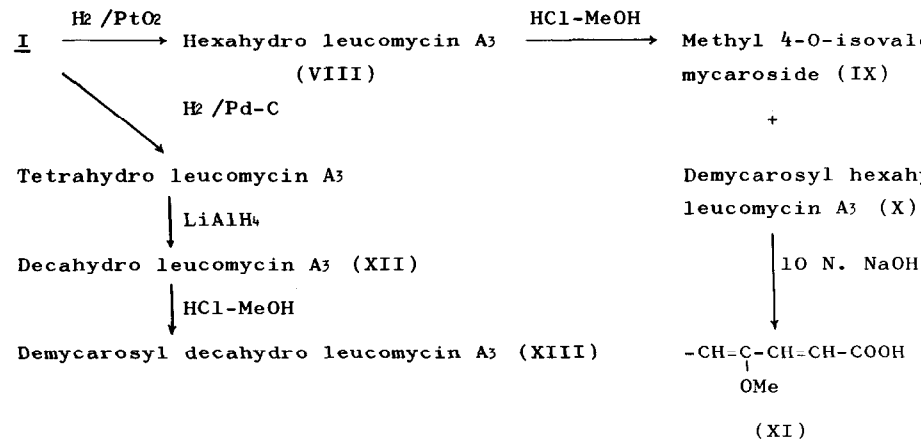
The position of $-CH_2CHO$ group of I[1] is confirmed at the neighboring position of the mycaminoside linkage by the method of Woodward[4]. As shown in the scheme, methyl demycarosyl leucomycin $A_3$ dimethylacetal (IV) is reduced by catalytic hydrogenation to give tetrahydro methyl demycarosyl leucomycin $A_3$[1], which is further reduced with $LiAlH_4$ to yield a triol (V). This aldehyde can be oxidized with $H_2O_2$ to afford an acid (VI), which upon heating with dil. HCl yields a γ-lactone (VII) showing an absorption at 1770 $cm^{-1}$ in the IR spectrum. These experiments enable one to confirm the position of he aldehyde group.




(IV) (V) (VII) (VI)

Hexahydro leucomycin A3 (VIII), $C_{42}H_{75}O_{15}N$, $[\alpha]_D$ -48.8° (c 1.35) is obtained by catalytic hydrogenation of I, and shows no aldehyde group in the IR and NMR spectra. Treatment of VIII with HCl in methanol yields methyl 4-O-isovaleryl mycaroside (IX) and demycarosyl hexahydro leucomycin A3 (X), $C_{30}H_{55}O_{11}N$, $[\alpha]_D$ -10.4° (c 1.33), and when the latter is warmed with 10 N. NaOH the acid (XI) is obtained as a colorless liquid, $\lambda_{max}^{0.01\ N\ NaOH}$ 265 mμ ($E_{1cm}^{1\%}$ 650), $\nu_{max}$ 1700, 1630 and 1605 $cm^{-1}$. The same alkaline treatment of hexahydroforocidine (obtained from spiramycin) gives an acid having the same UV spectrum, thus suggesting the presence of an α,β,γ,δ-unsaturated γ-methoxy acid system[4)5)] in XI. On the other hand, when decahydro leucomycin A3 (XII), $C_{35}H_{69}O_{13}N$, $[\alpha]_D$ -55.5° (c 1.33), obtained from tetrahydro leucomycin A3 by reduction with $LiAlH_4$, is submitted to the same HCl/MeOH treatment as described above for VIII, the demycarosyl derivative XIII, $C_{28}H_{57}O_{10}N$, $[\alpha]_D$ -15.0° (c 1.33), is obtained. Treatment of XIII with 10 N. NaOH fails to give an acid corresponding to the compound (XI), as evidenced by the UV spectrum. From these experiments, it is evident that the carbonyl group of the original lactone is converted to the carboxylic group of the acid (XI).

In the NMR spectrum of I (Fig. 1), the signal at 4.05 ppm., which is shifted to a lower field upon acetylation, can be assigned to the $C_9$ proton. The $C_9$ proton is coupled to the $C_{10}$ proton at 5.60 ppm. (J 8.9 cps) and the



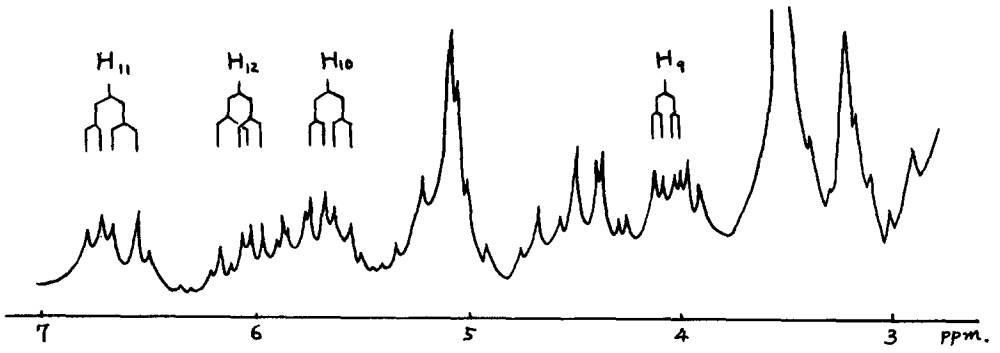
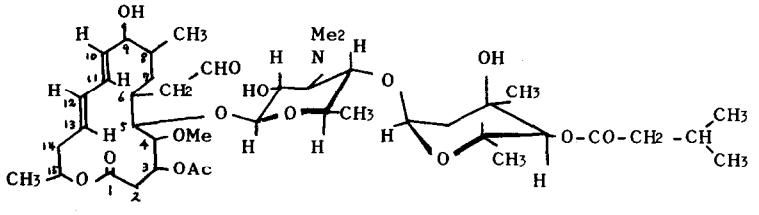



Fig. 1. NMR spectrum of leucomycin $A_3$ ($CDCl_3$ ; 100 Mc)

C8 proton (J 4.2 cps). Although the signal of the C8 proton cannot be detected, C8 should be a tertiary carbon from the quartet nature of the C9 proton peak. Signals of the C11 proton at 6.60 ppm. (1 H, $J_{11,10}$ 15.4 cps, $J_{11,12}$ 10.0 cps) and the C12 proton at 6.05 ppm. ($J_{12,11}$ 10.0 cps, $J_{12,13}$ 15.2 cps) in Fig. 1 indicate a trans-trans configuration for the diene system[6].

Finally, the stereochemistry of the glycoside linkage of leucomycin $A_3$ will be discussed. In the NMR spectra of I and related compounds, a 1 H doublet at 4.30 ppm. may be attributed to the anomer proton (1' H) (J 7.4 cps), and this suggests a β-configuration of the mycaminosidic linkage (Fig. 1). On the other hand, the stereochemistry of the mycaminoside linkage cannot be determied in a straight forward from the NMR, because many signals appear around 5.00 ppm. On the other hand, since diacetyl leucomycin $A_3$[1] has only one tertiary hydroxyl group, a comparison of the IR spectra in $CCl_4$ of the acetate and α- and β-methyl 4-O-isovaleryl mycaroside (IXa and IXb)[1] makes it possible to examine the formation of intramolecular hydrogen bonding. It is found that the β-isomer has an absorption peak at 3615 $cm^{-1}$ due to a free hydroxyl group. On the contrary the α-isomer has an absorption peak at 3530 $cm^{-1}$ which is due to a hydroxyl group hydrogen bonded to the glycosidic methoxyl oxygen. The hydroxyl group absorptions of acetyl leucomycin $A_3$, acetyl spiramycin and acetyl magnamycin appear at 3515, 3515, and 3518 $cm^{-1}$, respectively. It is therefore concluded that the mycarosidic

linkages of leucomycin A3, spiramycin and magnamycin adopt the α-forms. On the basis of these data, the structure shown below is proposed for leucomycin A3.



Leucomycin A3

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