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The Chemistry of Leucomycins. III.[1] Structure and Stereochemistry of Leucomycin A_3[2]

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Leucomycin A_3 (I) was oxidized with active manganese dioxide to dehydroleucomycin A_3 (II) which was identical with magnamycin B.

Acid treatment of I yielded demycarosylleucomycin A_3 (VII), which was converted to a triacetate showing mass spectrum M^+ (*m/e* 725), and this observation confirmed the structure of I. The diene system of I was assumed to have a *trans-trans* configuration on the basis of its NMR spectrum, and a full structure of I was proposed with stereochemistry.

It has already been reported[4,5] that leucomycin A_3 is composed of 4-O-[α-L-4-O-isovalerylmycaropyranosyl]-β-D-mycaminopyranoside and the macrolactone as an aglycone having groups such as $-CH_2CHO$, O-acetyl, O-methyl, and α,β,γ,δ-unsaturated alcohol or ether. Subsequent studies proved the full structure and stereochemistry of leucomycin A_3 in relation to magnamycin B.[6]

When leucomycin A_3 (I) is oxidized with active manganese dioxide[7] in chloroform, a dehydroleucomycin A_3 (II), $C_{42}H_{67}O_{15}N$, is obtained which shows an ultraviolet absorption maximum at 279.5 mμ. This result indicates that an α,β,γ,δ-unsaturated alcohol group is present in I and the alcohol is oxidized to the corresponding ketone[8] by manganese dioxide. Oxidation of an allylic hydroxyl group in the above reaction is also supported by the observation that dehydroleucomycin A_3 (II) gives only the monoacetyl derivative (III), $C_{44}H_{69}O_{16}N$. The infrared spectrum of II shows bands of α,β-unsaturated carbonyl at 1632 and 1595 cm^{-1}. The two compounds (II and III) are respectively identical with magnamycin B and its acetate[9,10] by comparison of their ultraviolet (UV), infrared (IR), and nuclear magnetic resonance (NMR) spectra, and the behavior on thin-layer chromatography. Magnamycin B is minor product of *Streptomyces halstedii*, together with magnamycin (carbomycin). From these experiments, the structure of I becomes celar as shown in Chart 1, and further support for the structure of I can be obtained from the following experiments.

When I is oxidized with ozone in methanol, an acid is obtained which was identified as β-hydroxybutyric acid on the basis of its NMR spectra and gas chromatographic comparison of its methyl ester with an authentic sample. This proves that I has the $CH_3-\underset{\underset{O}{\|}}{C}H-CH_2-CH=C<$ moiety.

1) Part II: *Chem. Pharm. Bull.* (Tokyo), **16**, 1167 (1968).
2) Abstract of papers, the 10th Symposium on the Chemistry of Natural Products, 1966, p. 86.
3) *a*),*b*) Location: *Shiba Shirogane Sankocho, Minato-ku, Tokyo.*
4) S. Ōmura, H. Ogura, and T. Hata, *Tetrahedron Letters*, **1967**, 609, 1267.
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6) R.L. Wagner, F.A. Hochstein, K. Murai, M. Messina, and P.P. Regna, *J. Am. Chem. Soc.*, **75**, 4684 (1953).
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9) F.A. Hochstein and K. Murai, *J. Am. Chem. Soc.*, **76**, 5080 (1954).
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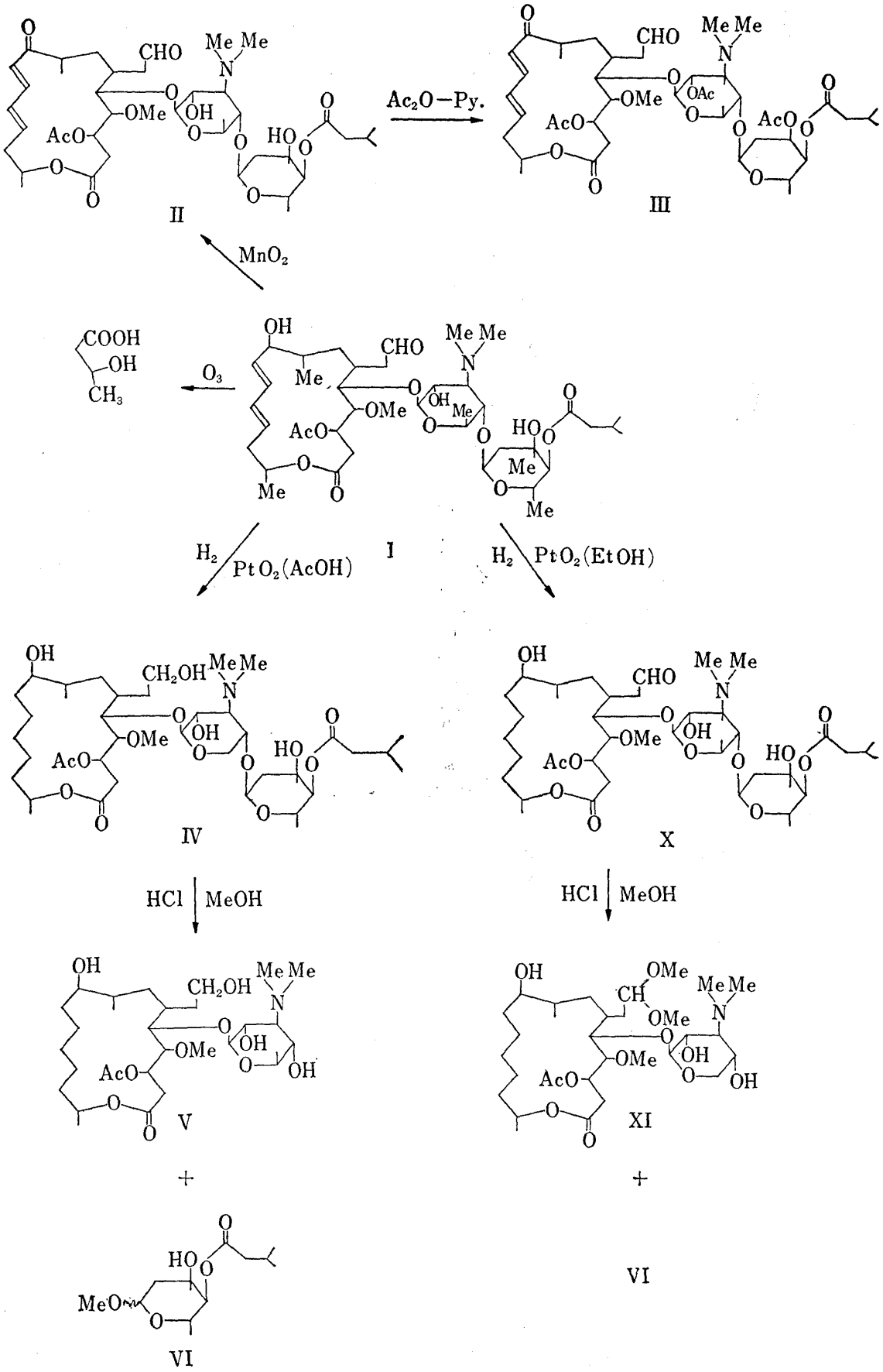


Chart 1

On the other hand, I is hydrogenated over platinum oxide in an acetic acid solution to give a hexahydro derivative (IV), which is hydrolyzed with hydrochloric acid to hexahydrodemycarosylleucomycin A_3 dimethylacetal (V) together with methyl 4-O-isovalerylmycaroside (VI). When V is refluxed with 10N sodium hydroxide, a colorless oily acid is obtained and chromatographed on silica gel. Although the acid is not obtained in a pure state, its ultraviolet spectrum shows an absorption peak at 265 mμ ($E^{1\%}_{1\,cm}$ 650) in 0.01N sodium hydroxide. The same alkaline treatment of hexahydroforocidine,[11] obtained from spiramycin, gives an acid having the same ultraviolet spectrum, suggesting the presence of a γ-methoxy-α,β,γ,δ-unsaturated acid system,[10,11] in this acid.

In the NMR spectrum of I (Fig. 1), the signal at 4.05 ppm, which shifts toa lower magnetic field upon acetylation can be assigned to the C_9 proton, by comparison with dehydroleucomycin A_3 (II). The C_9 proton is coupled to the olefinic proton (C_{10}) at 5.60 ppm with J=8.9 cps and the other proton (C_8) with J=4.2 cps. It seems reasonable from the value of couping constant to assign this to the C_8 proton, as it seems too large for the long-range coupling

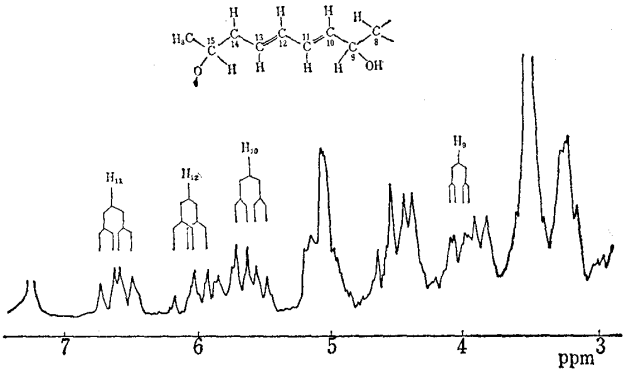
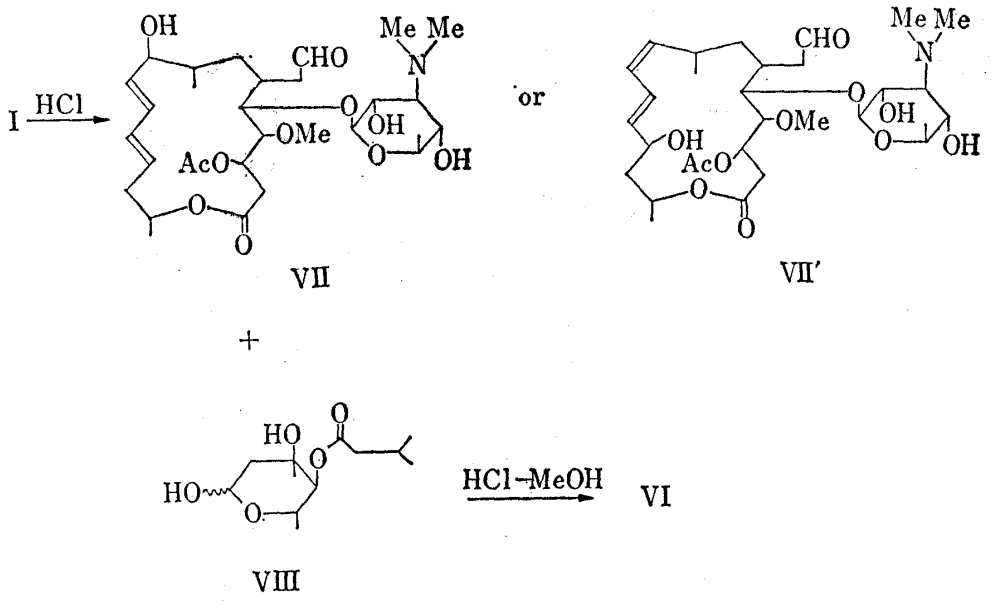


Fig. 1. NMR Spectrum of Leucomycin A_3($CDCl_3$, 100 Mc)



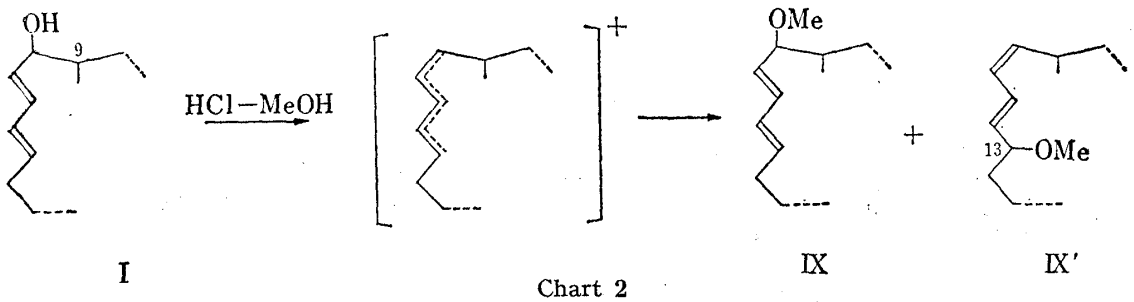


Chart 2

11) R. Paul and S. Tchelitcheff, *Bull. Soc. Chim. France*, 150 (1960).
12) J.W. Emsley, J. Feeney, and L.H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. 2, Pergamon Press, London, 1966, p. 710, 727.

with an olefinic proton at C_{11}.[12] Although the signal of the C_8 proton cannot be detected, C_8 should be a tertiary carbon from the quartet nature of the C_9 proton peak. Signals of the C_{11} proton at 6.60 ppm with the coupling constants of $J_{11,10}$=15.4 cps, $J_{11,12}$=10.0 cps, and the C_{12} proton at 6.05 ppm with the coupling constants of $J_{12,11}$=10.0 cps, $J_{12,13}$=15.2 cps in Fig. 1 indicate a *trans-trans* configuration for the diene system.[12]

I is hydrolyzed by diluted hydrochloric acid to a crystalline basic compound (VII), $C_{30}H_{49}O_{11}N$, and 4-O-isovalerylmycarose (VIII), $C_{12}H_{22}O_5$. The oily substance (VIII) is converted into methyl 4-O-isovalerylmycaroside[1,4,5] (VI) when treated with hydrochloric acid in methanol. On the other hand, the basic substance (VII) has a titration equivalent of 602±10 in 50% ethanol. Therefore, VII may be considered as demycarosylleucomycin A_3. Acetylation of VII by acetic anhydride in pyridine gives the corresponding triacetyl derivative, $C_{36}H_{55}O_{14}N$. The infrared spectrum of this triacetate in chloroform does not show absorption for a hydroxyl. The mass spectrum of the triacetate shows the presence of a molecular ion peak (*m/e* 725), and the above observation postulates that I has a molecular formula of $C_{42}H_{69}O_{15}N$ (molecular weight, 827).

It has been reported[4,5] the aldehyde group in I is converted into a dimethylacetal by treatment of I with methanolic hydrochloric acid, and one methoxyl group is newly introduced during this reaction. The resulting methyldemycarosylleucomycin A_3 dimethylacetal (IX) forms a diacetate.[4,5] On the other hand, demycarosylleucomycin A_3 (VII) forms a triacetate, and this difference indicates that one hydroxyl group is changed into a methoxyl group during methanolysis. This is further supported by the fact that of tetrahydroleucomycin A_3[4,5] (X) gives to tetrahydroleucomycin A_3 dimethylacetal (XI) which forms a triacetate (XII). In the NMR spectrum of XII (Fig. 2), two methoxyl groups of the dimethylacetal are observed at 3.1 and 3.2 ppm besides the original methoxyl absorption at 3.5 ppm.

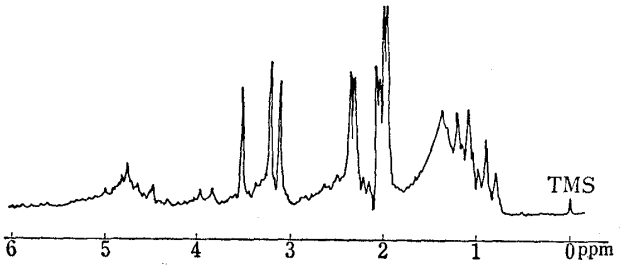
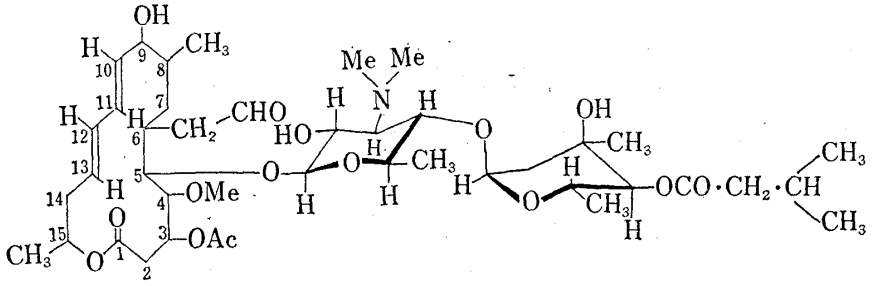


Fig. 2. NMR Spectrum of Triacetyldemycarosyltetrahydroleucomycin A_3 Dimethyl Acetal ($CDCl_3$, 60 Mc)

The reaction mechanism of this hydrolysis or methanolysis of I[2] is not yet clear, an allylic rearrangement[13] may occur during such a reaction. The original hydroxyl group of I should be present at C_9 from the above-cited NMR studies, though the hydrolyzed compounds (VII and IX) have a hydroxyl group (VII) or methoxyl group (IX) at C_9 or C_{13} through the allylic rearrangement from C_9. In conclusion, the formulae of the hydrolyzed compounds (VII and X) should be indicated respectively by VII or VII′ and IX or IX′. This problem is now under investigation in our laboratories.



Leucomycin A_3 (I)

Chart 3

13) P. de Mayo, "Molecular Rearrangements," Vol. I, Interscience Publishers, New York, 1963, p. 27.

Experimental[14]

Dehydroleucomycin A_3 (II)——One gram of leucomycin A_3 (I) was dissolved in 35 ml of $CHCl_3$ and 10 g of activated MnO_2[7] was added. After removal of the solvent, the residue was chromatographed on silicic acid (Mallinkrodt). The eluates of benzene–Me_2CO (3:1) were examined by thin-layer chromatography (TLC) (benzene–Me_2CO (1:1)). From the first eluate, 0.20 g of II was obtained and purified by the recrystallyzation from Me_2CO–H_2O (5:1), mp 140—141° (decomp.), $[\alpha]_D^{25}$ −34.0° (c=1.0). UV λ_{max}^{MeOH} mμ (ε): 279.5 (21900). pK_a 6.71 (50% EtOH). Violatile acid 2.1 moles. *Anal.* Calcd. for $C_{42}H_{67}O_{15}N$: C, 61.07; H, 8.18; N, 1.70. Found: C, 61.35; H, 8.22; N, 1.65.

This compound was identical with magnamycin B in its NMR, IR, and UV spectra and behavior on TLC.

Acetyldehydroleucomycin A_3 **(III)**——The acetate (III) was prepared from 0.20 g of II in 2 ml of pyridine and 0.2 ml of Ac_2O. The crude oily product was chromatographed on 6 g of silicic acid and eluted by benzene–Me_2CO (10:1) to give III as white powder (0.18 g). After recrystallization from Me_2CO–H_2O (5:1), 0.08 g of white needles were obtained. mp 148—150°, $[\alpha]_D^{25}$ −50.0° (c=1.0), pK_a 5.70 (50% EtOH). Volatile acid, 3.0 moles. *Anal.* Calcd. for $C_{44}H_{69}O_{16}N$: C, 60.88; H, 8.01; N, 1.61. Found: C, 61.00; H, 8.11; N, 1.70.

This acetate was identical with acetylmagnamycin B in its NMR and IR spectra, and the behavior on TLC.

Ozonolysis of Leucomycin A_3 **(I)**——Ozonolysis of I (2.0 g) in 50 ml of MeOH at −60° followed by decomposition of the ozonide with H_2O_2 produced acidic products (0.30 g). The NMR spectra of the acidic products in $CDCl_3$ showed characteristic signals for β-hydroxybutyric acid and isovaleric acid at 0.98 ppm (d, J=7.0 cps, $-CH\cdot\underline{Me}_2$), 2.22 ppm (d, $CH\cdot C\underline{H}_2$-COOH) (isovaleric acid), 1.25 ppm (d, J=7.0 cps, $-\underset{\underset{OH}{|}}{CH}\cdot\underline{Me}$), 2.50 ppm (d, J=7.0 cps. $-\underset{\underset{OH}{|}}{CH}\cdot C\underline{H}_2$-COOH), and 4.25 ppm (m, $-CH_2-\underset{\underset{OH}{|}}{C\underline{H}}\cdot Me$) ($\beta$-hydroxybutyric acid). A mixture of the acidic products was esterified with CH_2N_2 and gas-chromatographed over SE-30 (20%). The two peaks obtained were identified with authentic samples of methyl β-hydroxybutyrate and methyl isovalerate.

Hexahydroleucomycin A_3 **(IV)**——Leucomycin A_3 (I) (1.0 g) in AcOH (50 ml) was hydrogenated over PtO_2 until the absorption of H_2 had ceased. After 3 moles of H_2 was absorbed, the filtrate was evaporated under a reduced pressure, the residual syrup was dissolved in 20 ml of H_2O, pH adjusted to 8.0 with dil. NaOH, and extracted with $CHCl_3$. After evaporation of the dried solution, the residue was chromatographed over silicic acid with benzene–Me_2CO (4:1). There was obtained 0.80 g of IV as fine powder from ether–petr. ether. $[\alpha]_D^{25}$ −48.8° (c=1.3). *Anal.* Calcd. for $C_{42}H_{75}O_{15}N$: C, 60.48; H, 9.06; N, 1.68. Found: C, 60.55; H, 9.10; N, 1.70.

Methanolysis of Hexahydroleucomycin A_3 **(IV)**——One gram of IV was dissolved in 1% HCl in MeOH, kept for 6 hr at room temperature, and neutralized to pH 4 with dil. NaOH. The neutralized mixture was concentrated under a reduced pressure to remove MeOH, diluted with H_2O, and extracted with ether. Methyl 4-O-isovalerylmycaroside (VI) (0.20 g) was obtained from ether extract as a liquid, bp 115—118° (1 mmHg). This was identified with an authentic sample[5] by comparison of their NMR spectra. The aqueous layer was adjusted to pH 8.5 with dil. NaOH and extracted with $CHCl_3$. After removal of the solvent from the extract, the remaining basic material was dissolved in a minimum amount of ether and 2 ml of petr. ether was added. There was obtained 0.65 g of hexahydrodemycarosylleucomycin A_3 (V) as a fine white powder, $[\alpha]_D^{25}$ −10.4° (c=1.4). *Anal.* Calcd. for $C_{30}H_{55}O_{11}N$: C, 59.48; H, 9.15; N, 2.31. Found: C, 59.80; H, 9.20; N, 2.32.

Alkali Treatment of Hexahydrodemycarosylleucomycin A_3 **(V)**——V (0.50 g) was added to 3 ml of 10N NaOH and the turbid solution was refluxed on an oil bath for 2 hr. The oily layer was separated from the aqueous layer by salting outed, the oily layer was dissolved in 2 ml of H_2O, and acidified with 1N HCl. The acid solution was extracted with $CHCl_3$. The remaining oily material, which was obtained from the dried $CHCl_3$ solution, was chromatographed over silicic acid with MeOH–benzene (1:1) and 0.15 g of a viscous liquid was obtained. UV $\lambda_{max}^{0.01\,N\,NaOH}m\mu$ ($E_{1cm}^{1\%}$): 265 (650).

Methanolysis of Tetrahydroleucomycin A_3 **(X)**——By treatment of 0.50 g of X[4,5] with HCl–MeOH, as described above, 0.25 g of tetrahydrodemycarosylleucomycin A_3 dimethylacetal (XI) was obtained as a fine powder. *Anal.* Calcd. for $C_{32}H_{59}O_{12}N$: C, 59.15; H, 9.15; N, 2.16. Found: C, 59.80; H, 9.21; N, 2.22.

From the neutral portion, methyl 4-O-isovalerylmycaroside (VI) (0.06 g) was obtained.

Triacetyltetrahydrodemycarosylleucomycin A_3 **Dimethyl-1-Acetal**——Acetylation of 0.2 g of XI was carried out as described above and 0.15 g of amorphous powder (one spot on TLC) was obtained. *Anal.* Calcd. for $C_{38}H_{65}O_{15}N$: C, 58.82; H, 8.44; N, 1.81. Found: C, 59.11; H, 8.50; N, 1.91.

14) All temperatures are uncorrected. Unless otherwise stated rotations were measured in $CHCl_3$. Elemental analyses were carried out by the Microanalytical Laboratory, Kitasato University.

Acid Hydrolysis of Leucomycin A_3 (I)——A solution of 5 g of I dissolved in 60 ml of 0.35N HCl was allowed to stand for 20 hr at 5°. After adjusting to pH 4 with dil. NaOH, the reaction mixture was extracted with $CHCl_3$ and the extract was dried. After removal of the solvent from the extract, 4-O-isovalerylmycarose (VIII) was obtained as a greenish syrup. This was used for the following experiment without purification. The aqueous layer was made alkaline with dil. NaOH and extracted with $CHCl_3$ which was dried and evaporated. The residue was crystallized from benzene and recrystallized from CCl_4 to 1.0 g of demycarosylleucomycin A_3 (VII) as needles, mp 199—202° (decomp.), $[\alpha]_D^{25}$ −14.0° (c=1.0). Titration in 50% EtOH showed an equivalent weight of 602±10, pK_a 7.80. Volatile acid, 1.0 mole. *Anal.* Calcd. for $C_{30}H_{49}O_{11}N$: C, 60.08; H, 8.24; N, 2.34. Found: C, 60.50; H, 8.17; N, 2.28.

Methyl 4-O-Isovalerylmycaroside (VI)——One gram of the crude VIII was dissolved in 50 ml of 1% HCl in MeOH. The solution was refluxed for 2 hr and treated as described in methanolysis of I[1,5] to give 0.80 g of VI as a colorless liquid, bp 120—122° (1.5 mmHg). *Anal.* Calcd. for $C_{13}H_{24}O_5$: C, 59.98; H, 9.29. Found: C, 60.15; H, 9.36.

This material was identified with an authentic sample[4,5] of VIII by its IR and NMR spectra.

Acetyldemycarosylleucomycin A_3——VII (0.50 g) was acetylated as described above and 0.45 g of the acetate of VII was obtained. Recrystallization from EtOH–H_2O (10:1) gave the acetate as needles, mp 195—196°, pK_a 5.35 (50% EtOH). Volatile acid, 3.89 moles. M^+ 725 m/e. *Anal.* Calcd. for $C_{36}H_{55}O_{14}N$: C, 59.57; H, 7.64; N, 1.93. Found: C, 60.00; H, 7.82; N, 1.99.

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