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196. Satoshi Ōmura, Michiko Katagiri,*1 Haruo Ogura,*2 and Toju Hata*1,2 : The Chemistry of Leucomycins. I. Partial Structure of Leucomycin A_3.*3

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Leucomycin A_3 is a new antibiotic which has been isolated along leucomycin A_1 and other components, from the fermentation broth of *Streptomyces Kitasatoensis* HATA. The present paper is concerned with the partial structure of leucomycin A_3 as a macrolide antibiotic and composed of a lactone containing aldehyde, O-acetyl and O-methyl groups, mycaminose, and 4-O-isovaleryl mycarose.

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Leucomycins have been found to be one of the macrolide antibiotics isolated from *Streptomyces Kitasatoensis* HATA by T. Hata, *et al.*[1] Leucomycin A_1,A_2 and B_1, B_2, B_3 and B_4 have been already reported as components of leucomycins.[2] Recently we isolated a new component, named leucomycin A_3, from a crude leucomycin bulk and studied on the chemical structure.[3]

In the course of chemical degradative and spectroscopical studies, leucomycin A_1 has proved to have a carbonyl group, a conjugated double bond, and an epoxide by Watanabe, *et al.*[4] Leucomycin A_3 can be separated by chromatography on a column of silicic acid eluted by benzene-acetone, and crystallized from benzene as colorless prisms, m.p. 120~121°, and the possible formula, $C_{42}H_{69}O_{15}N$ (mol. wt. 827) is compatible with the elemental analyses and molecular weight determinations (osmometry, 850±25 (chloroform) titration, 835±10 (50% ethanol)). This molecular formula is also compatible with the elemental analyses and mass spectra of several derivatives and degradation products of leucomycin A_3.

The antibacterial spectrum of leucomycin A_3 (Table I) is closely resemble to that of leucomycin A_1.[5] The LD_{50} for mice is 580 mg./kg. by an intravenous injection method.

Although leucomycin A_3 (I) contains one nitrogen atom, negative ninhydrin and Van Slyke nitrogen tests, indicates the absence of any primary amine. Tollen's and tetrazolium tests are positive. The unsaturated character of I is indicated by decolorization with permanganate and bromine. Zeizel test shows the presence of one methoxyl group. Alkaline hydrolysis of I gives the sodium salts of acetic acid and isovaleric acid, which compared with authentic samples by paper chromatography[6] and the nuclear magnetic resonance (NMR) spectra.

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*3 Presented before the 10th Symposium on the Chemistry of Natural Products, p. 86 (1966).

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Table I. Minimum Inhibitory Concentration of Leucomycin A_1 and A_3

	mg./ml., 24 hr.	
	A_1	A_3
St. aureus FDA 209 P	0.04	0.15
St. aureus LM EMR	10	10
B. subtilis PCI 219	0.3	0.6
Mycoba. avium	10	10
St. hemolyticus	0.08	0.15
D. pneumoniae II	0.02	0.08
C. diphtheriae	0.04	0.04
N. gonarrhoeae	0.3	0.6
H. influenzae	0.08	0.15
K. pneumoniae PCI 602	5	10
S. typhsamurium	10	10
E. Coli NIHJ	10	10

Method: Agar dilution in Bouillon agar.

The infrared spectrum of I shows strong peaks at 1728 and 1746 cm^{-1} (carbonyl), 1230 cm^{-1} (acetyl), and weak absorption band at 2725 cm^{-1} (aldehyde) and 1661 cm^{-1} (double bond). The NMR spectrum of I in deuterochloroform is shown in Fig. 1. The spectrum suggests the presence of one dimethylamino group (6H, s, 2.49 p.p.m.), one methoxyl group (3H, s, 3.47 p.p.m.), one aldehyde group (1H, s, 9.56 p.p.m.), one acetyl group (3H, s, 2.22 p.p.m.), four olefinic protons (4H, m, 5.3~6.7 p.p.m.) and a number of C-methyl groups at around 1 p.p.m.

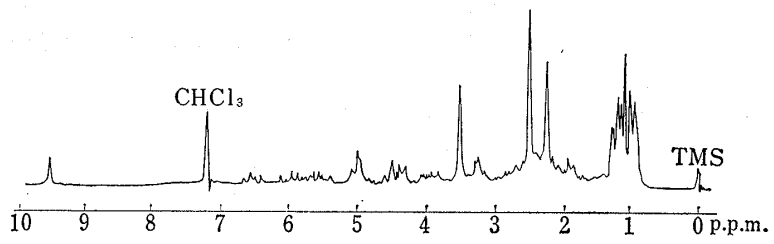


Fig. 1. NMR Spectrum of Leucomycin A_3 ($CDCl_3$, 100 Mc)

The ultraviolet absorption spectrum of I shows a strong peak at 231.5 mμ (ε 29100) as shown in Fig. 2. The position and intensity of the peak are characteristic of a α, β, γ, δ-unsaturated alcohol or an ether group.[7]

The ultraviolet absorption maxima of the thiosemicarbazone (III) are observed at 232 mμ (ε 36315) and 271.5 mμ (ε 24330) as shown in Fig. 2, characteristic of saturated thiosemicarbazone.[8] The NMR spectrum of III in acetone indicates a triplet at 7.62 p.p.m. (1H, J=5.0 c.p.s.) due to the proton of the group, -CH=N-, and those facts suggest the presence of $-CH_2CHO$ group*4 in I.

A crystalline diacetyl derivative (II) is formed on acetylation in acetic anhydride-pyridine. The remaining hydroxyl group is observed in II by the infrared spectrum, indicating a presence of a tertiary hydro-

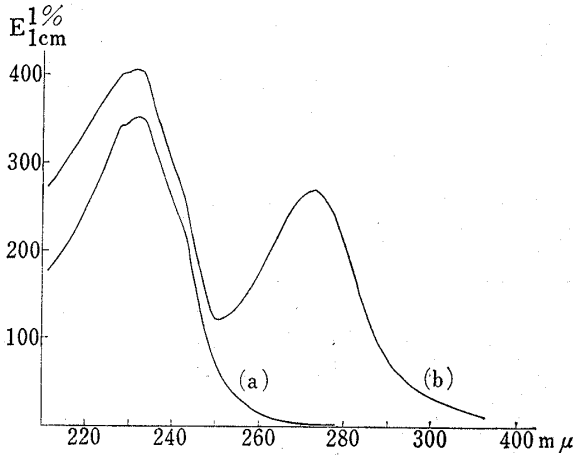


Fig. 2. Ultraviolet Absorption Spectra of Leucomycin A_3 (a) and Its Thiosemicarbazone(b)

*4 Unpublished work.

7) M.E. Kuehne, B.W. Benson: J. Am. Chem. Soc., **87**, 4660 (1965). R. Paul, S. Tchelitcheff: Bull. soc. chim. France, 1059 (1957).

8) D.Y. Curtin, J.A. Gourse, W.H. Richardson, K.L. Rinehart, Jr.: J. Org. Chem., **24**, 93 (1959).

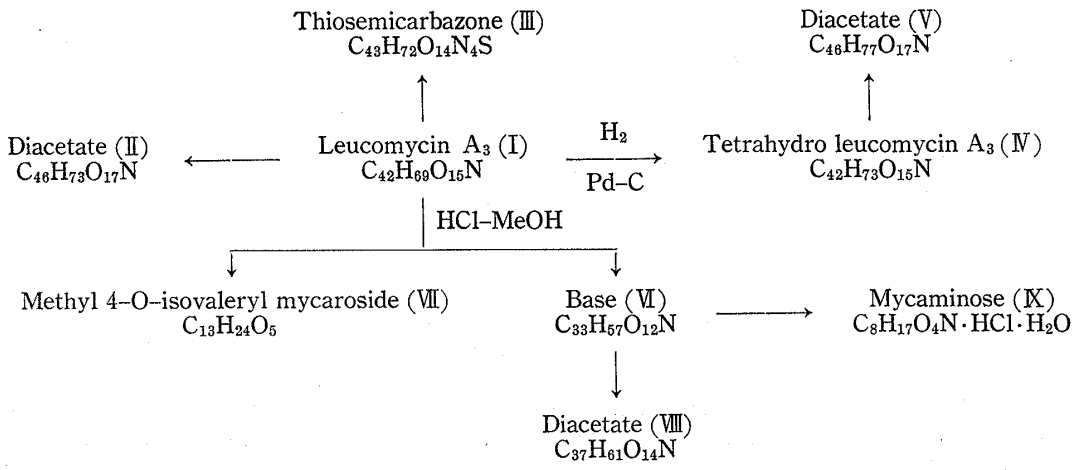


Chart 1.

xyl or sterically hindered hydroxyl group in I. The hydrogenation of I over palladium charcoal results the prompt absorption of two moles of hydrogen and yields the tetrahydro derivative (Ⅳ) which is converted to the corresponding diacetate (Ⅴ) with acetic anhydride-pyridine. The infrared absorption band at 1661 cm^{-1} and the ultraviolet absorption maximum of I are disappeared by the hydrogenation.

Methanolysis of I by methanolic hydrochloric acid yields an amorphous base (Ⅵ) and an oily neutral substance (Ⅶ). The neutral material is identified as methyl 4-O-isovalerylmycaroside (methyl 4-O-isovaleryl-2,6-didesoxy-3-C-methyl-L-ribohexose) through comparison of its infrared spectrum with that of the authentic specimen obtained from leucomycin A_1.[2] Acetylation of Ⅵ with acetic anhydride-pyridine yields a corresponding crystalline diacetate (Ⅷ). The infrared spectrum of Ⅷ, in carbontetrachloride indicates the absence of hydroxyl absorption. The NMR spectra of Ⅵ and Ⅷ show the existence of a dimethyl acetal group (3.15, 3.25 p.p.m.) and the absence of an aldehyde group absorption in Ⅵ, and one methoxyl group in addition to the acetal group is observed in Ⅷ as shown in Fig. 3. The NMR spectra of Ⅷ in deuterochloroform and benzene indicate the presence of three C-methyl groups.

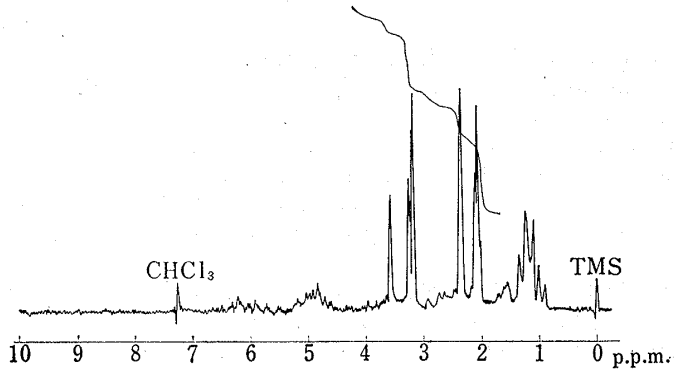
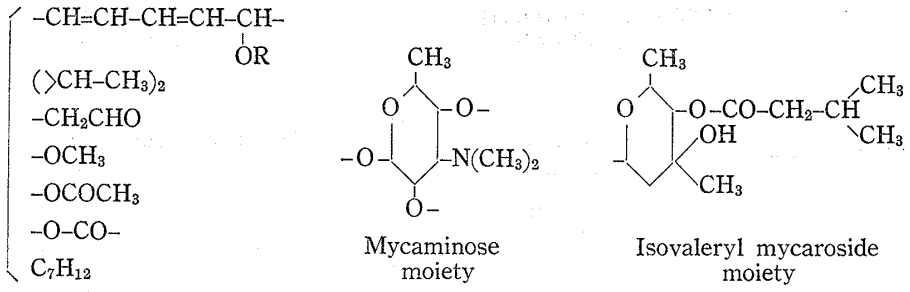


Fig. 3. NMR Spectrum of Diacetyl-methyl-demycarosyl-leucomycin A_3 Dimethyl Acetal ($CDCl_3$, 60 Mc)

By vigorous acid hydrolysis of Ⅵ an amino sugar, mycaminose (3,6-didesoxy-3-dimethylamino-D-glucopyranose), is obtained as a hydrochloride (Ⅸ), which is identified by comparison of its infrared spectrum, optical rotation and mixed melting points with an authentic sample yielding from spiramycin.[9] Therefore one of three tertiary methyl groups which are observed in the NMR spectra of Ⅵ and Ⅷ, should be belonged to mycaminose.

Ⅵ is treated with a dilute alkaline solution to obtain an amphoteric substance by hydrolysis of the lactone ring that suggest mycaminose is attached to the macrolactone moiety. On the basis of these evidences, it is concluded that leucomycin A_3 have following partial structures.

9) R. Paul, S. Tchelitcheff: Bull. soc. chim. France, 734 (1957).



Experimental*5

Leucomycin A_3 (I)——In order to obtain single component, various chromatographic absorbents such as alumina and silicic acid, were tried with a leucomycin bulk. In these methods the chromatography with silicic acid was found to be suitable.

The bulk (10 g.) was dissolved in benzene and subjected to chromatography with a column of 300 g. of silicic acid (Mallinkrodt 100 mesh powder). The active component was eluted with benzene-acetone (5:1). The eluate was fractionated each 15 ml. portion and each fraction was examined for antimicrobial activity, and the thin-layer chromatography with Kieselgel G and benzene-acetone (1:1) as a developing solvent.

The first eluate was proved to contain weak active noncrystalline impurities and following eluate was shown to contain leucomycin A_3. After removing the solvent from the eluate containing I, the residue was crystallized from benzene, yielding 1 g. (10%) leucomycin A_3 (I) as colorless prisms, m.p. 120~121°, UV λ_{max} mμ ($E^{1\%}_{1cm.}$) : 231.5 (351), $[\alpha]^{25}_{D}$ −58.0° (c=5.0, MeOH), pKa′ 6.70±2. *Anal.* Calcd. for $C_{42}H_{69}O_{15}N$: C, 60.93; H, 8.40; N, 1.69. Found : C, 60.57; H, 8.19; N, 1.75.

Diacetyl-leucomycin A_3 (II)——I (1.5 g.) was acetylated with 10 ml. pyridine containing 2 ml. acetic anhydride. Recrystallization from CCl_4 gave needles, m.p. 125~126°. *Anal.* Calcd. for $C_{46}H_{73}O_{17}N$: C, 60.57; H, 8.07; N, 1.54. Found : C, 60.65; H, 8.10; N, 1.60.

Thiosemicarbazone of I (III)——I(1.2 g.) and thiosemicarbazide (0.135 g.) were dissolved in 18 ml. of EtOH. The solution was heated under reflux for 4 hr. After filtration, the solvent was evaporated to dryness and extracted in $CHCl_3$. After recrystallization of the extract, there was obtained 0.6 g. (46%) of III, m.p. 138~141°, UV λ_{max} mμ ($E^{1\%}_{1cm.}$) : 232 (403), 271.5 (270). *Anal.* Calcd. for $C_{43}H_{72}O_{14}N_4S$: C, 57.32; H, 8.05; N, 6.22; S, 3.56. Found : C, 56.97; H, 8.00; N, 6.25; S, 3.60.

Tetrahydro-leucomycin A_3 (IV)——I (3.0 g.) was hydrogenated over 0.5 g. of 5% Pd-C in 150 ml. of EtOH at 25°. During a period of 2 hr., the reaction mixture absorbed 1.98 molar equivalents of hydrogen. The filtrate evaporated to dryness, and triturated in ether and petroleum ether to give 2.9 g. (97%) of IV as a white finely divided powder. *Anal.* Calcd. for $C_{42}H_{73}O_{15}N$: C, 60.63; H, 8.84; N, 1.68. Found : C, 60.57; H, 8.62; N, 1.70.

Diacetyl-tetrahydro-leucomycin A_3 (V)——One g. of IV was acetylated as above II to give 0.50 g. (45%) of V as white needles (from CCl_4-hexane, 5:1), m.p. 115~118°, $[\alpha^{25}_{D}]$ −74.0° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{46}H_{77}O_{17}N$: C, 60.31; H, 8.47; N, 1.53. Found : C, 60.41; H, 8.35; N, 1.61. Volatile acid 3.9 mol.

Alkaline Hydrolysis of I——I (0.200 g.) was dissolved in 9 ml. of EtOH and added 1.00 ml. of 1.00 *N* KOH. After refluxing for 30 min. the solution was titrated with 0.10 *N* HCl (2.95 molar equivalents of alkaline was consumed). After evaporation of EtOH, remaining aqueous solution was acidified with 10 ml. of 30% phosphoric acid, and subjected to steam distillation (1.9 molar equivalents of volatile acids were obtained). The neutralized distillate with NaOH was concentrated to dryness and extracted with $CHCl_3$, the remained sodium salts were determined as acetic acid and isovaleric acid on the paper chromatography[6] and the NMR spectrum.

Methanolysis of I——I (12.5 g.) was dissolved in 80 ml. of MeOH and 7 ml. of c. HCl and the solution was allowed to stand for 18 hr. at 4°, then neutralized to pH 4 with dil. NaOH. The neutralized mixture was concentrated under reduced pressure to remove MeOH and extracted with ether. The ether extract gave 2.5 g. of VII as a liquid, $b.p_1$ 115~116°. M^+ 260 m/e.

The oily material was identified through comparison of its IR spectrum with methyl 4-O-isovaleryl-mycaroside obtained from leucomycin A_1.[2]

The remaining aqueous layer was neutralized with dil. NaOH and extracted with $CHCl_3$. Evaporation of the solvent from the dried solution yielded 7.5 g. of the basic material, which was chromatographed on

*5 Nuclear magnetic resonance spectra of 60 Mc were measured with Hitachi H-60 spectrometer and 100 Mc were measured with Varian HA-100 spectrometer. Unless otherwise specified, infrared spectra were measured in KBr tablet with Nihonbunko IR-S spectrometer, and ultraviolet spectra were measured in methanol. Temperatures were uncorrected. Microanalyses were carried out by Central Laboratory of the Kitasato University, and the data were given as a mean value of two or three runs.

silica gel to obtain methyl demycarosyl-leucomycin A_3 dimethyl acetal (Ⅵ, 6.0 g.), as a white powder, $[\alpha]_D^{25}$ $-18.4°$ (c=1.5, $CHCl_3$), UV λ_{max} mμ ($E_{1cm}^{1\%}$): 232 (426). *Anal.* Calcd. for $C_{33}H_{57}O_{12}N$: C, 60.07; H, 8.71; N, 2.12. Found: C, 60.23; H, 8.37; N, 2.18. Volatile acid 1.1 mol.

Acetate——This was obtained in the same manner as described above, m.p. 179~181°, $[\alpha]_D^{25}$ $-10.5°$ (c=1.3, $CHCl_3$), M^+ 317 m/e. *Anal.* Calcd. for $C_{37}H_{61}O_{14}N$: C, 59.66; H, 8.39; N, 1.88. Found: C, 60.00; H, 8.40; N, 1.90.

Acid Hydrolysis of VI——Three grams of Ⅵ was dissolved in 40 ml. of 3% HCl and heated under reflux for 2 hrs. After removing the insoluble material, the dark filtrate was washed with $CHCl_3$, and evaporated to dryness under reduced pressure. The residue was extracted with 10 ml. of hot iso-PrOH and neutralized with dil. NaOH, cooled and filtrated. Concentration of the filtrate, there was obtained mycaminose-HCl-H_2O (Ⅸ, 0.85 g., 75.8%) as colorless prisms. Recrystallization from hot 96% iso-PrOH showed m.p. 113~116°. Mixed m.p. was 113~115° with an authentic sample from spiramycin.[9] *Anal.* Calcd. for $C_8H_{17}O_4N \cdot HCl \cdot H_2O$: C, 39.10; H, 8.21; N, 5.71. Found: C, 39.15; H, 8.21; N, 5.67.

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