THE CHEMISTRY OF LEUCOMYCINS. IV

STRUCTURE OF LEUCOMYCIN A_1

SATOSHI ŌMURA, MICHIKO KATAGIRI and TOJU HATA

Kitasato Institute, Minato-ku, Tokyo, Japan

(Received for publication October 6, 1967)

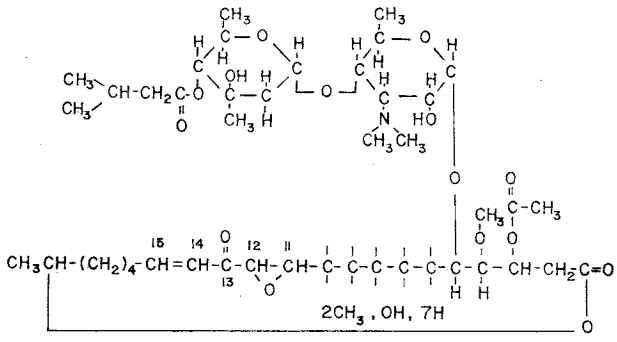
Leucomycin A_1 is the main and most active component of leucomycins. From the degradative and comparative studies of the component with leucomycin A_3, which is a new component of leucomycins, the proposed structure for leucomycin A_1 is revised.

The structure of leucomycin A_1 which is the main and most active component of the leucomycins was the subject of a brief communication[1]. We now report the details, plus some spectroscopic data for leucomycin and its acetate.

Structural studies on the antibiotic were carried out by WATANABE *et al.*[2] and it was characterized as a macrolide antibiotic having a 4-O-isovaleryl mycarose, one O-methyl, one O-acetyl and one carbonyl group conjugated between an epoxide and carbon-carbon double bond as shown below (Ia).

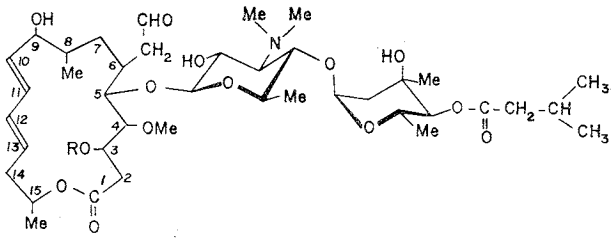
Recently, CELMER[3] called attention to the abnormality of the structure in that the biogenetic feature of the partial structure at C_{11}~C_{15} on a large membered lactone (Ia) does not fit into a normal concept of other macrolides, such as magnamycin and spiramycin.

Formula 1. Ia



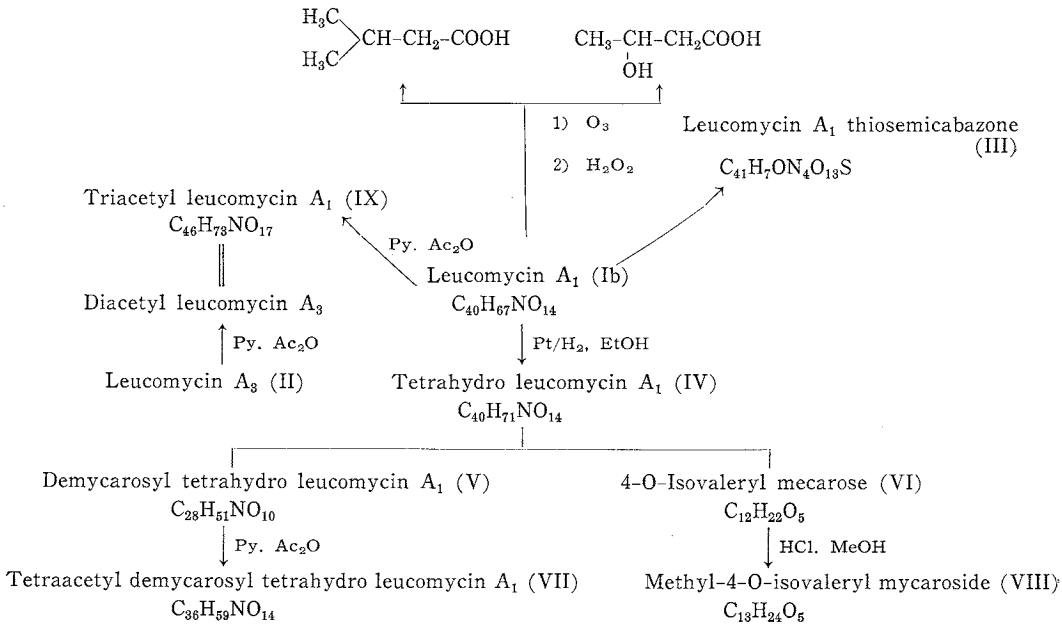
Structure II was previously proposed for leucomycin A_3[4,5,6] which is a new component isolated from the leucomycin complex. From the comparative studies of leucomycin A_1 with leucomycin A_3 the earlier proposed structure of leucomycin A_1 was revised to Ib.

Formula 2. Ib : R=H, II : CO-CH_3



Leucomycin A_1 (Ib) was isolated from the leucomycin complex by use of chromatography over both silicic acid and aluminum oxide[7]. Several attempts to crystallize of Ib from solvents, such as benzene and ethanol (or acetone)-water were unsuccessful, but the homogeneity could be confirmed with thin layer chromatography over

Scheme I.



both Kiesel Gel G (benzene-acetone, 1 : 1) and aluminum oxide (ethyl acetate).

The molecular formula, $C_{46}H_{81}NO_{17}$[1] of leucomycin A_1 previously reported, was revised to $C_{40}H_{67}NO_{14}$ from the elemental analysis and molecular weight determination; titration method 809 ± 10, vapour pressure method 800 ± 15, and elemental analysis of nitrogen 785 ± 15. This formula also is compatible with the elemental analysis of its crystalline derivatives.

The physicochemical properties of leucomycin A_1, ultraviolet absorption, pKa', and relative rotation are very similar to those of leucomycin A_3.

The presence of the acetyl group in the structure Ia was not allowed by the IR and NMR spectra in which the acetate absorption was not observed.

On alkaline hydrolysis of Ib, 2 moles of alkali were consumed and one mole of volatile acid was titrated. The volatile acid was identified as isovaleric acid by the NMR spectrum of the sodium salt in D_2O. Leucomycin A_1 (Ib) was converted to the thiosemicarbazone (III).

Hydrogenation of Ib over platinum catalyst in ethanol yielded the tetrahydrate (IV). Ozonolysis of Ib in methanol and oxidation of the resulting ozonide with hydrogen peroxide in alkaline medium yielded β-hydroxy butyric acid and isovaleric acid. Hydrolysis of IV with a 0.5 N HCl yielded an amorphous base (V) and 4-O-isovaleryl mycarose which was converted to the methylglycoside (VIII) by refluxing with 1 % hydrochloric acid-methanol.

Leucomycin A_1 (Ib) and amorphous base (V) were converted to the corresponding triacetate and tetraacetate respectively. From these findings leucomycin A_1 is expected to have the same structure as leucomycin A_3 after allowing for the replacement of the O-acetyl group at C-3 of leucomycin A_3 by the hydroxyl group.

It has been found that leucomycin A_1 triacetate and leucomycin A_3 diacetate have

Fig. 1. NMR spectrum of triacetyl leucomycin A_1 ($CDCl_3$, 100 Mc/s)

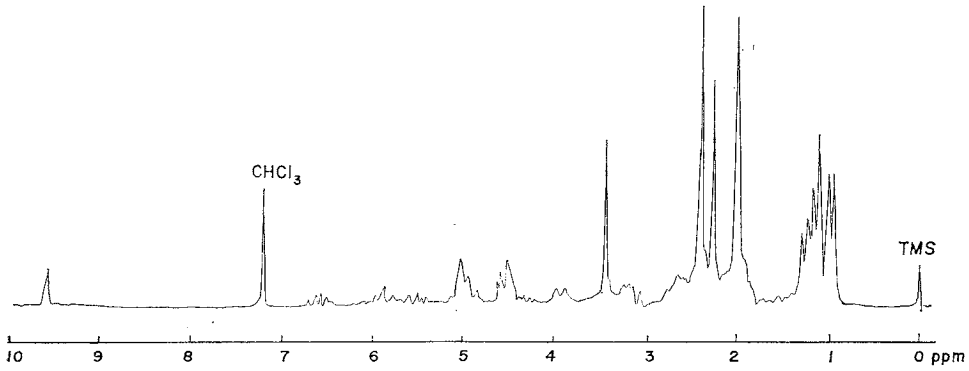
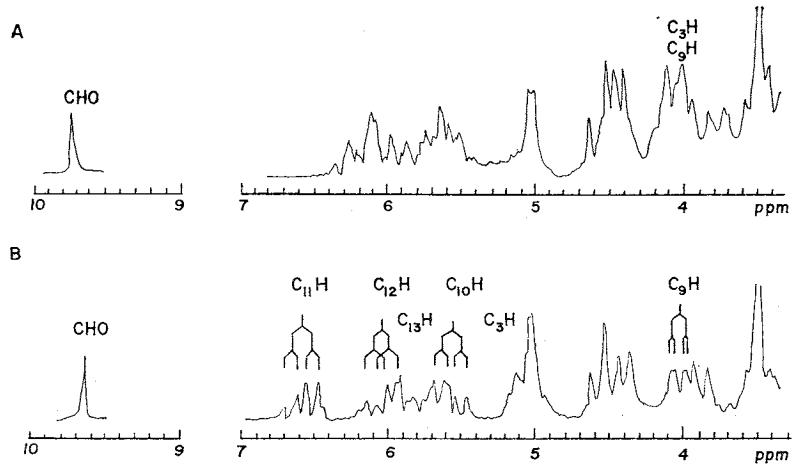


Fig. 2. NMR spectra of leucomycin A_1 (A) and leucomycin A_3 (B) ($CDCl_3$, 100 Mc/s)



the same structure by comparison of the NMR (Fig. 1), IR spectra, and behavior on thin layer chromatography.

Some characteristic changes in the NMR spectra of leucomycin A_1 (Ib) and leucomycin A_3 (II)[4,6] were observed as shown in Fig. 2. First of all, the singlet aldehyde methine absorption of Ib was shifted from 9.78 ppm to 9.65 ppm upon the replacement of the hydroxyl group at C-3 by the O-acetyl group. Considerable changes in the absorption pattern of olefinic protons were observed in the comparison of NMR spectra of Ib and II. Above all, the C-11 proton was isolated from the other three olefinic protons to lower magnetic fields in the Ib. On the basis of these spectra, it seemed reasonable to assume that acetylation on C-3 had an important influence on the conformation of the other partial structure of lactone.

Experimental

The isolation of leucomycin A_1 will be described in another article[7] together with that of the other components of leucomycin[1,2].

The physicochemical properties of leucomycin A_1 are as follows; $[\alpha]_D^{25}$ −66.0 (c 1.0, $CHCl_3$), pKa′ 6.69 (50 % EtOH), UVλ_{max}^{MeOH} 232 mμ ($E_{1\%}^{1cm}$ 400), mol. wt. 809±10 (titration method), 800±15 (vapor pressure method), 785±15 (elemental analysis of nitrogen).

Anal. Calcd. for $C_{40}H_{67}NO_{14}$: C 61.11, H 8.60, N 1.78
Found : C 61.36, H 8.50, N 1.70.

Leucomycin A_1 thiosemicarbazone (III)

Leucomycin A_1 (1.5 g) and thiosemicarbazide (0.15 g) were dissolved in 18 ml of ethanol. The solution was refluxed on a water bath for 3 hours and after the mixture was allowed to stand in ice for 2 hours, the crystalline product was filtered and washed with cold ethanol. The product was recrystallized twice from ethanol and dried at 80°C *in vacuo*. m. p, 150~152°C (decomp.), UV λ_{max}^{MeOH} mμ ($E_{1cm}^{1\%}$) : 232 (418), 272 (281).

Anal. Calcd. for $C_{41}H_{71}N_4O_{13}S$: C 57.32, H 8.21, N 6.52, S 3.73
Found : C 56.88, H 7.85, N 6.41, S 3.56.

Tetrahydro leucomycin A_1 (IV)

Leucomycin A_1 (2 g) was hydrogenated over PtO_2 catalyst (100 mg) in ethanol (60 ml) at atmospheric pressure. The uptake of two equivalents of hydrogen was observed within one hour.

The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in ether and then petroleum ether was added. The solution was concentrated to dryness, giving a white powder.

Anal. Calcd. for $C_{40}H_{71}NO_{14}$: C 60.81, H 9.06, N 1.77
Found : C 60.95, H 9.09, N 1.75.

Acid hydrolysis of tetrahydro leucomycin A_1

Tetrahydro leucomycin A_1 (1.5 g) was dissolved in 30 ml of 0.5 N HCl and allowed to stand at room temperature for 4 hours. The reaction mixture was first extracted with ether and the aqueous layer was adjusted to pH 8.5 with a diluted solution of sodium hydroxide. The basic solution was then extracted with chloroform, and the extract was concentracted to dryness. The residue was dissolved in ether and treated with petroleum ether to give 1.2 g of demycarosyl tetrahydro leucomycin A_1 (V), which was acetylated as follows:

The oily material from the ether extract was dissolved in methanol containing 1 % hydrochloric acid and the solution was refluxed to give methyl 4-O-isovaleryl mycaroside (VIII), b. p. 115~120°C/2 mmHg.

Anal. Calcd. for $C_{13}H_{24}O_5$: C 59.98, H 9.29
Found : C 60.01, H 9.20.

Tetraacetyl demycarosyl tetrahydro leucomycin A_1 (VII)

The powder (IV) (1.0 g) was acetylated with pyridine-acetic anhydride. The acetate was crystallized from 90 % ethanol to give needles, m. p. 138~140°C, $[\alpha]_D^{25}$ −10.5 (c 1.0, $CHCl_3$).

Anal. Calcd. for $C_{36}H_{59}NO_{14}$: C 59.24, H 8.15, N 1.92
Found : C 59.35, H 8.20, N 1.93.

Ozonolysis of leucomycin A_1

Leucomycin A_1 (3 g) was ozonolysed in 50 ml of methanol. After decomposition of the ozonide with hydrogen peroxide in alkaline medium, the acidic fraction obtained was extracted with ethyl acetate. The crude extract (400 mg) was analysed without further purification.

The acidic product (20 mg) was dissolved in ether and the solution was esterified with diazomethane and gas-chromatographed over SE-30 (20 %/Silicon gum 6.5×100 cm). The resulting two peaks were identified with an authentic sample as methyl β-hydroxy butyrate and methyl isovalerate.

The characteristic signals of β-hydroxy butyric acid and isovaleric acid were observed on the NMR spectra of the acidic products in $CDCl_3$ as follows:

0.98 ppm (d, J=7.0 cps, $CH\langle^{CH_3}_{CH_3}$), 2.22 ppm (d, $>CHCH_2$-COOH).

1.25 ppm (d, J=7.0 cps, $\underset{\underset{OH}{|}}{CH}-\underset{=}{CH_3}$), 2.50 ppm (d, J=7.0 cps, $\underset{\underset{OH}{|}}{CH}-\underset{=}{CH_2}-COOH$),

4.25 ppm (m, $\underset{\underset{OH}{|}}{\underset{=}{CH_2}}CHCH_3$)

Triacetyl leucomycin A_1 (IX)

Leucomycin A_1 (1 g) was acetylated with pyridine-acetic anhydride. The product was crystallized from carbon tetrachloride and recrystallized from the same solvent to give 700 mg of needles, m. p. 124~126°C, $[\alpha]_D^{25}$ −82.5 (c 1.3, $CHCl_3$), pKa′ 5.72 (50 % EtOH).

Anal. Calcd. for $C_{46}H_{73}NO_{17}$: C 60.58, H 8.07, N 1.54
Found : C 60.70, H 8.12, N 1.60.

The NMR spectra (Fig. 1), IR spectra, melting point, and behavior on thin-layer chromatography of the triacetate obtained thus were identical with those of leucomycin A_3 diacetate.

Acknowledgement

The authors wish to thank Dr. H. OGURA, Kitasato University and Dr. K. NAYA, Kansaigakuin University, for stimulating discussion, and Dr. J. ABE and T. WATANABE, Toyō Jozō Co., Ltd. for their kind supply of leucomycin complex.

References

1) HATA, T.; S. ŌMURA, M. KATAGIRI, H. OGURA, K. NAYA, J. ABE & T. WATANABE : Structure of leucomycin A_1. Chem. Pharm. Bull. 15 : 358~359, 1967
2) WATANABE, T.; T. FUJII, H. SAKURAI, J. ABE & K. SATAKE : Structure of leucomycin. IUPAC Symposium of the Chemistry of Natural Products. p. 145, 1964
3) CELMER, W. D. : Biogenetic, constitutional, and stereochemical unitary principles in macrolide antibiotics. Antimicr. Agents & Chemoth. 1965 : 144~156, 1966
4) HATA, T.; S. ŌMURA, A. MATSUMAE, M. KATAGIRI & Y. SANO : Leucomycin A_3, a new antibiotic from *Streptomyces kitasatoensis*. Antimicr. Agents & Chemoth. 1966 : 631~636, 1967
5) ŌMURA, S.; H. OGURA & T. HATA : The chemistry of the leucomycins. I. Partial structure of leucomycin A_3. Tetrahedron Letters No. 7 : 609~613, 1967
6) ŌMURA, S.; H. OGURA & T. HATA : The chemistry of the leucomycins. II. Structure and stereochemistry of leucomycin A_3. Tetrahedron Letters No. 14 : 1267~1271, 1967
7) ŌMURA, S.; M. KATAGIRI, K. MIYAZAKI, S. SATO K. TSUCHIDA, T. TAKE, T. WATANABE, J. ABE & T. HATA : The chemistry of leucomycins. V. Isolation of new components from leucomycin complex. J. Antibiotics (in press).