# ISOLATION AND STRUCTURE OF SF-1902 $A_5$, A NEW GLOBOMYCIN ANALOGUE

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SF-1902 is a solvent-soluble, neutral antibiotic produced by *Streptomyces hygroscopicus* SF-1902[1]. It shows antibacterial activity only against Gram-negative bacteria[2]. HPLC analysis of the sample seemingly homogeneous on TLC (silica gel, chloroform – methanol, 5: 1, or acetone – benzene, 2: 1) disclosed the presence of several minor components and one major component SF-1902, as shown in Fig. 1. Those were separated from each other by using a semi-preparative LC column of $\mu$Bondapak $C_{18}$. Among these, SF-1902 and SF-1902 $A_5$ were obtained as crystals. When 850 mg of the crude sample of SF-1902 was purified by recrystallization and semi-preparative LC, 530 mg of SF-1902, 32 mg of $A_2$, 100 mg of $A_3$, 50 mg of $A_4$ and 75 mg of $A_5$ were obtained. SF-1902, a major component, was identified with globomycin[3] by the direct comparison including ORD curves in chloroform. Among these minor components, only $A_5$ was obtained as homogeneous crystals. Further purification and the structural studies of other minor components are now under study.

Fig. 1. HPLC analysis of SF-1902 preparation
Column: $\mu$Bondapak $C_{18}$ (8 mm × 300 mm)
Solvent: Acetonitrile-water, 60: 40
Flow rate: 1.7 ml/min.

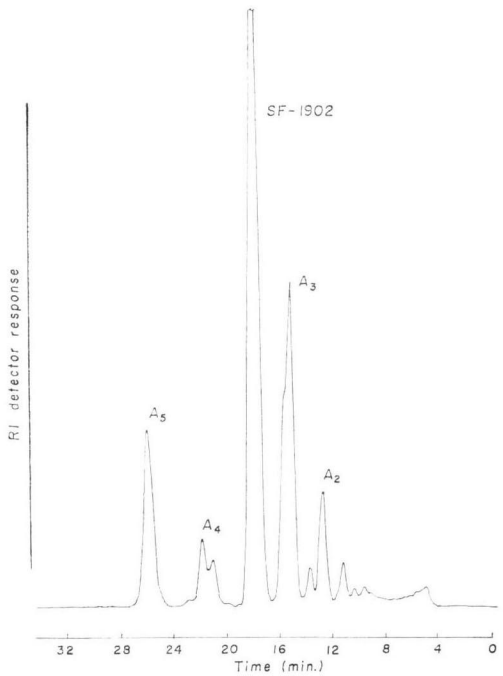



Fig. 2. IR spectrum of SF-1902 $A_5$ (KBr)

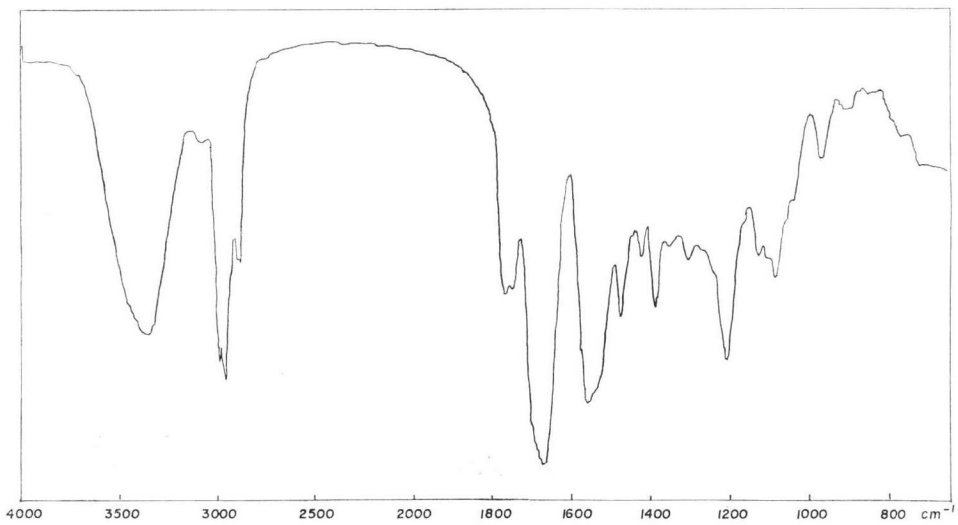
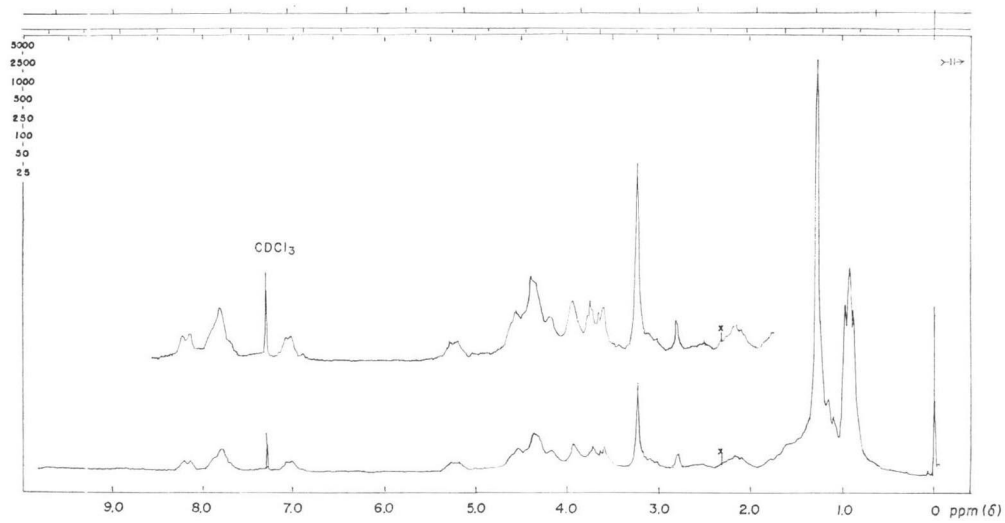


Fig. 3. 100 MHz PMR spectrum of SF-1902 $A_5$ in $CDCl_3$




Physico-chemical Properties of SF-1902 $A_5$ (I)

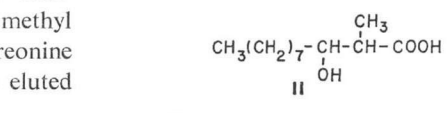
SF-1902 $A_5$ (I) was separated only by HPLC, but not by TLC so far. It shows solubility very similar to SF-1902. I was obtained as fine needles from 60% aqueous acetonitrile and posessed the following physico-chemical characteristics: m.p. 113~117°C, $[\alpha]_D^{20}$ −16° (*c* 0.7, $CHCl_3$) (after drying at 70°C *in vacuo* for 10 hours), PMR (Fig. 2, $CDCl_3$), 3.2 ppm (NMe), MS $M^+$ 683 ($C_{34}H_{61}N_5O_9$), Anal. Calcd. for $C_{34}H_{61}N_5O_9$: C, 59.71; H, 8.99; N, 10.24% Found: C, 59.85; H, 8.85; N, 10.30%; IR (Fig. 3, KBr), 2960, 2930, 1740, 1650 and 1540 $cm^{-1}$, very similar to that of SF-1902.

Components of I

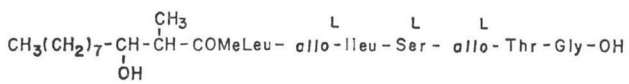
Acid hydrolysis followed by the Dowex 50WX2 chromatography (0.1 M pyridine-acetate buffer, pH 3.1) gave each one mole of L-*allo*-threonine, L-serine, glycine, N-methylleucine and L-*allo*-isoleucine. These amino acid components were identical with those of globomycin. Each of them was identified by PPC, amino acid analysis, PMR and optical rotation. *allo*-Threonine structure was confirmed by comparing its retention time on GLC as its methyl ester N-trifluoroacetate with those of threonine and *allo*-threonine. *allo*-Threonine was eluted in 3.2 minutes, while threonine was eluted in 2.2 minutes (0.75% OV-1 on Gas-Chrom Q 1.2 m, 85°C, He flow rate: 2.2 ml/min.). From these data, SF-1902 $A_5$ (I) is considered to be a homologue of globomycin. CMR spectrum of I in $CDCl_3$ is indistinguishable from that of globomycin except for 28~32 ppm region indicating the presence of extra 2 methylene units. GC-MS analysis of the fatty acid moiety (II) of I, as its methyl ester acetate, gave a molecular ion at *m/e* 272, indicative of an original molecular formula of II as $C_{12}H_{24}O_3$ (retention time; 6.1 minutes, $RT_{nonadecane}$; 0.76). The structure of II was finally confirmed to be *threo*-3-hydroxy-2-methylundecanoic acid by GC and NMR comparisons[4,5] of it and the synthetic reference sample of *threo* and *erythro* isomers prepared from nonanal and 2-bromopropionic acid by the REFORMATSKY reaction according to BELLARSOUED *et al.*[6].

Structure of I

The amino acid sequence was determined by the application of mass spectrometry and found to be identical with that of globomycin. Alkali treatment of I gave an open chain compound (III), which was permethylated.

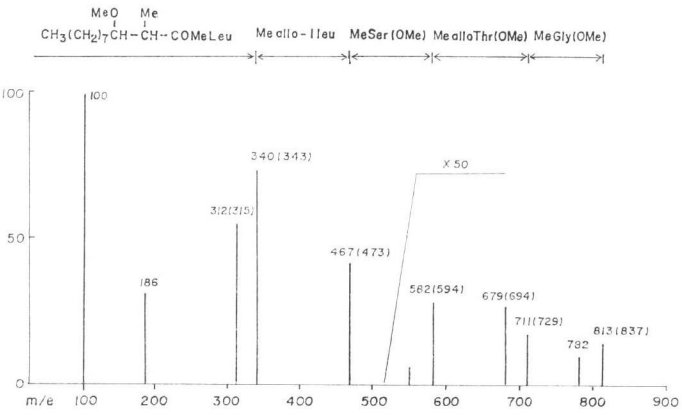



II




III

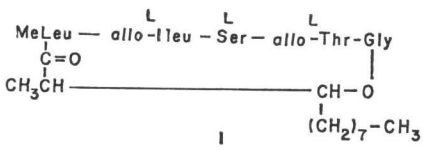
Fig. 4. The mass spectrum of the permethylated and the perdeuteromethylated (parentheses) derivatives of III




The mass spectrum of the permethylated derivative of **III** (Fig. 4) gave a molecular ion at *m/e* 813 accompanied by the peaks at *m/e* 711, 582, 467 and 340 derived from the cleavage at the peptide bonds. The peak at *m/e* 340 indicated that the acyl group attached to N-methylleucine was $C_{12}$-carboxylic acid instead of $C_{10}$-carboxylic acid in globomycin. Since the perdeuteromethylated analogue of **III** gave N-acyl-methylleucine fragment at *m/e* 343 accompanied by the peak at *m/e* 473 for N-methylleucyl-*allo*-isoleucine fragment, the sequence of N-acyl-N-methylleucyl-*allo*-isoleucine was clearly determined.

The carboxyl group of glycine should be bound to one of the hydroxyl groups to form a lactone ring, since **I** is neutral. The site of lactone ring formation was shown to be identical with that of globomycin because 50~80 ppm region of CMR was superimposable with that of globomycin. Assignment of these CMR signals has been carried out by the application of known chemical shift data of amino acids[7] and off resonance data. In the CMR of **I**, 7 signals are observed in the region of 50~80 ppm in $CDCl_3$. Of these, 6 signals from 56.6 to 68.5 ppm can be assigned to the following carbons: 56.6 ppm: *allo*-Ileu-$\alpha$; 58.5 ppm: Ser-$\alpha$; 59.2 ppm: *allo*-Thr-$\alpha$; 61.3 ppm: Ser-$\beta$; 66.8 ppm: *allo*-Thr-$\beta$; 68.5 ppm: Me-Leu-$\alpha$. The downfield shifts of $\alpha$-carbons of serine and *allo*-threonine with the upfield shifts of $\beta$-carbons of these amino acids on acetylation indicated that the hydroxyl groups of these amino acids were free in **I**. Furthermore, the signal at 76.7 ppm can be assigned to C-3 of 3-hydroxy-2-methylundecanoic acid in which the hydroxyl group has been esterified with the carboxyl group of glycine to form a lactone ring. Since C-3 of **II** is observed at 73.4 ppm in $CDCl_3$, it is rational to assign the signal at 76.7 ppm in **I** to the signal of C-3 of **II**. The downfield shift by 3.3 ppm in **I** of this signal strongly suggests that the hydroxyl group of **II** is esterified with the carboxyl group of glycine to form a lactone ring. These results lead to the structure **II** for SF-1902 $A_5$.

SF-1902 $A_5$ shows antibacterial activity stronger than globomycin. Details of its bioactivity will be reported in a subsequent paper discussing the structure-activity relationship of SF-1902 complex.

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