SF-1961, A NEW ANTIBIOTIC RELATED TO BLEOMYCIN

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A new type of bleomycin group antibiotic, SF-1961 was isolated from the fermentation broth of *Streptomyces* species. SF-1961 possessed β-amino-β-(4-amino-6-carboxypyrimidin-2-yl)-propionic acid instead of its 5-methyl derivative which was a common component of bleomycins and phleomycins.

In our screening studies of new antibiotics, a novel bleomycin group antibiotic was isolated from the culture filtrate of *Streptomyces* sp. SF-1961, which was isolated from a soil sample collected at Himeji, Hyogo, Japan. In this paper, taxonomy of the strain, isolation and characterization of the antibiotic are described.

Taxonomic Studies

The taxonomic characterization of the strain SF-1961 was carried out by the same method as described in the preceding paper[1]. The microorganism was identified as a strain of *Streptomyces filamentosus*[2,3] possessing following characteristics: Spore-chain morphology is classified in the *Rectiflexibilis* section. Spores were cylindrical to oval, 0.4~0.6 by 0.9~1.4 μ in size, with smooth surface. Mature aerial mass color was in the Red color-series on most agar media. Reverse side of colony did not show distinctive pigment. Melanoid pigment was not formed. Glucose, fructose, mannitol, inositol, arabinose, xylose, rhamnose and raffinose were utilized for growth. This strain has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan as FERM-P 3940.

Fermentation

A well-grown agar slant of the producing organism was used to inoculate to the seed medium containing 2.0% soluble starch, 1.0% Polypeptone, 0.3% beef extract and 0.05% K_2HPO_4. After incubation for 20 hours at 28°C, the first seed culture (1%) was inoculated into five shaking flasks each containing 100 ml of medium to prepare the second seed culture. The second seed culture was then transferred into a 30-liter jar fermentor for 20 hours, 10 liters of the third culture were seeded into 200 liters of medium containing 2.5% glycerol, 2.5% soybean meal, 0.5% fish meal, 0.25% NaCl and 0.0005% $CuSO_4 \cdot 5H_2O$ in a 300-liter tank. The fermentation was carried out at 28°C for 20 hours and then at 24°C for an additional 70 hours.

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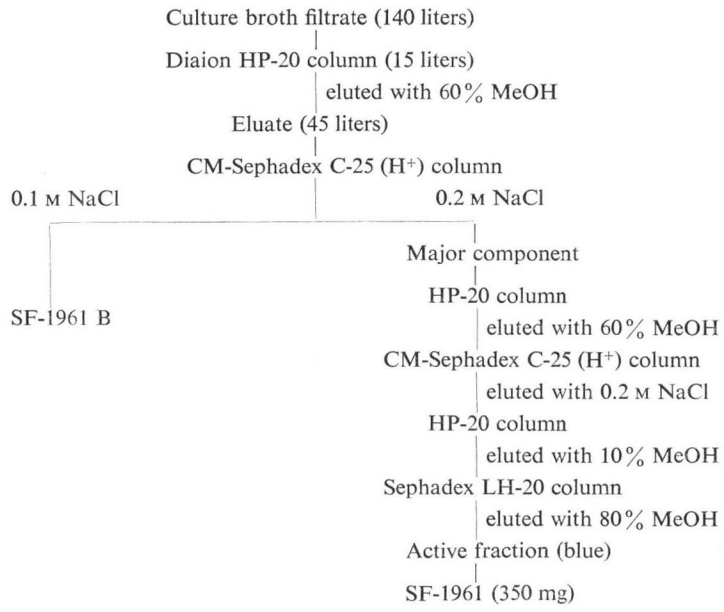
Chart 1. Isolation and purification of copper-chelated SF-1961.



Isolation and Purification

Isolation and purification of SF-1961 are summarized in Chart 1. Isolation was effected by employing the HP-20 and CM-Sephadex columns successively. We obtained a minor component SF-1961 B in addition to SF-1961; however, the amount of the minor component was so small that there was little information on the minor component.

Physico-chemical Properties

Physico-chemical properties of SF-1961 are summarized in Table 1. The copper-chelated form of SF-1961 was obtained as a blue powder, readily soluble in water and methanol. SF-1961 gave positive color reactions with GREIG-LEABACK and EHRLICH, but was negative to ninhydrin and SAKAGUCHI reactions. UV maxima of SF-1961 along with its IR spectrum (Fig. 1, 1020~1100, 1365, 1520, 1660, 1720 and 3300 cm^{-1}) suggested that SF-1961 was closely related to bleomycin group antibiotics. Although UV maxima of SF-1961 were slightly deviated from those in bleomycin, the ratio (*ca.* 1.4) of absorbancies at 250 nm and 286 nm for SF-1961 suggested the presence of a bleomycin type chromophore rather than phleomycin type. SF-1961 B showed very close properties showing UV maxima at 250 nm ($E^{1\%}_{1cm}$ 173) and 290~293 nm ($E^{1\%}_{1cm}$ 126) and showing very similar IR spectrum to that of SF-1961.

Table 1. Physico-chemical properties of copper-chelated SF-1961 hydrochloride.

Appearance	Blue powder
m.p.	203°C (dec.)
UV max (in water)	250 nm ($E^{1\%}_{1cm}$ 157.6), 286 nm ($E^{1\%}_{1cm}$ 111)
Microanalysis	C: 41.70, H: 5.58, N: 12.66, S: 3.68, Cl: 2.84, Cu: 3.10%
Solubility	
Soluble	Water, methanol
Insoluble	Chloroform, acetone, benzene
Color reaction	
Positive	EHRLICH, potassium permanganate, GREIG-LEABACK
Negative	Ninhydrin and SAKAGUCHI

Fig. 1. IR spectrum of SF-1961 hydrochloride (KBr pellet).

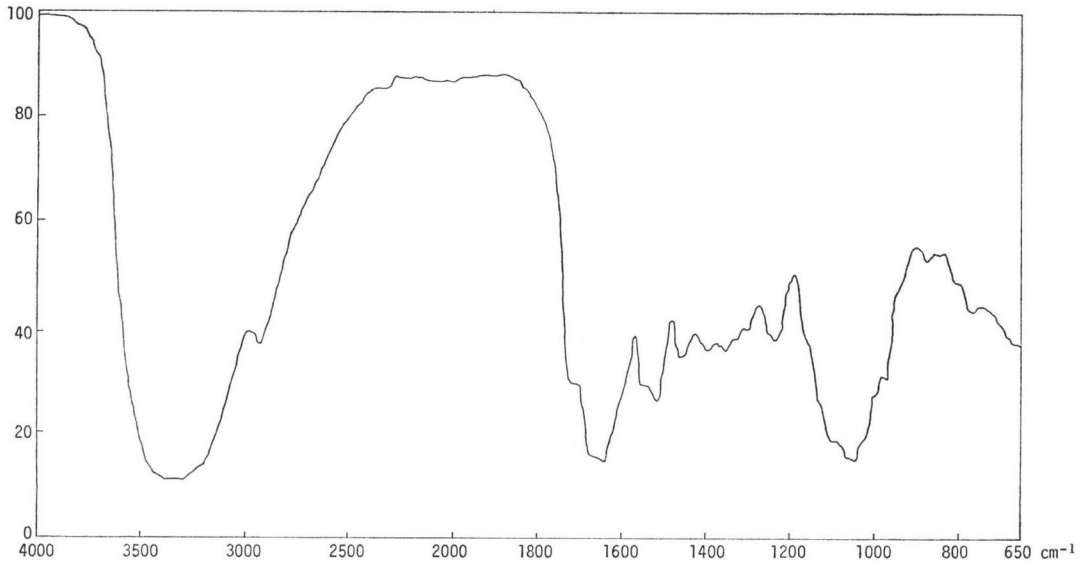
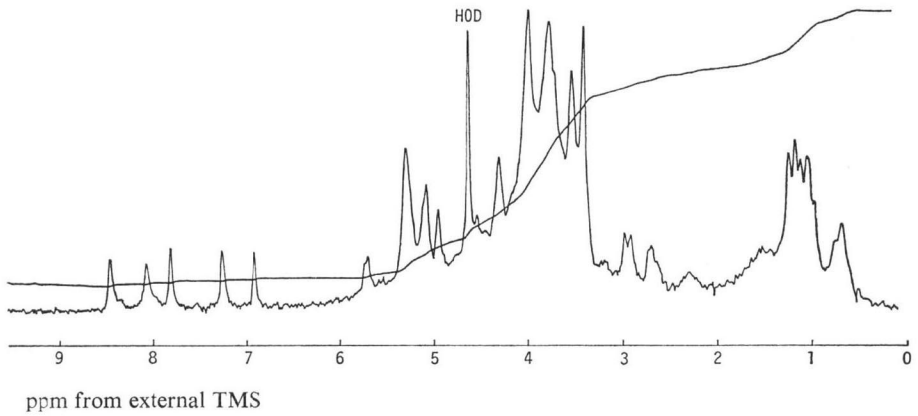


Fig. 2. PMR spectrum of SF-1961 (100 MHz, D_2O).



Biological Properties

Table 2 summarizes the antimicrobial spectrum of copper-free SF-1961. It showed strong antibacterial activities against some selected *Escherichia coli* and *Salmonella* strains. Antibacterial activities of the minor component was almost equal or slightly weaker than SF-1961.

Discussion

The physico-chemical properties of SF-1961 described above indicated close similarities to those of phleomycin and bleomycin group antibiotics. Until now, many bleomycin-phleomycin group antibiotics have been isolated, however, detailed structure studies were carried out only on bleomycin[4], phleomycin[4], YA-56[5], tallysomycin[6] and cleomycin[7].

Table 2. Antimicrobial spectrum of copper-free SF-1961.

Test organisms	MIC (mcg/ml)	Test organisms	MIC (mcg/ml)
Staphylococcus aureus JC-1	25	*Salmonella typhimurium* LT-2	0.39
Staphylococcus aureus S-424	100	*Salmonella enteritidis* No. 11	0.78
Staphylococcus epidermidis ATCC 14990	12.5	*Sarcina lutea*	3.13
Staphylococcus epidermidis 109	12.5	*Shigella sonnei* EW 33 Type 1	0.78
Streptococcus faecalis ATCC 8043	>100	*Klebsiella pneumoniae* PCI 602	0.78
Bacillus anthracis No. 119	50	*Klebsiella pneumoniae* 22#3038	100
Escherichia coli JC-2	0.78	*Proteus vulgaris* OX 19	6.25
Escherichia coli No. 29	6.25	*Proteus rettgeri* J-0026	100
Escherichia coli RGN 823	0.39	*Proteus morganii* Kono	25
Escherichia coli JR 66/W 677	50	*Serratia marcescens* MB-3848	>100
Citrobacter freundii GN 346 (CSase; H)	0.78	*Pseudomonas aeruginosa* MB-3829	>100
Salmonella typhi 0-901-W	0.39	*Pseudomonas cepacia* M-0527	12.5

Amino acid components

As a preliminary study for the chemical structure of SF-1961, amino acid components and sugar components were examined using acid hydrolyzate and methanolyzate of the copper-free sample. Two dimensional cellulose TLC combined with high voltage electrophoresis revealed the presence of six ninhydrin positive components. Based on the relative mobility and color reaction, four components were readily identified as threonine (**I**)*, 4-amino-3-hydroxy-2-methyl-*n*-valeric acid (**III**)*, β-hydroxyhistidine (**IV**)* and β-aminoalanine (**V**)* which were common components in bleomycin group antibiotics. β-Amino-β-(4-amino-6-carboxy-5-methylpyrimidin-2-yl)-propionic acid (**II**) was not detected. Although 2′-(2-aminoethyl)-2,4′-bithiazole-4-carboxylic acid (**VI**)* could not be detected in the acid hydrolyzate, however, PMR-spectrum of SF-1961 (Fig. 2) showed the signals of the bithiazole ring system in the lowest field region. KONISHI *et al.*[6] reported that bithiazole component could not be detected or isolated by drastic acid hydrolysis when this component had an unstable side chain at the 2′ position. Therefore, the bithiazole component in SF-1961 was considered to be 2′-polyfunctionally substituted bithiazole carboxylic acid (**VI′**) as found in tallysomycin. Of particular significance was that β-amino-β-(4-amino-6-carboxypyrimidin-2-yl)-propionic acid (demethyl-**II**), which was identified by the direct TLC comparison with the authentic sample[8], was found in the acid hydrolyzate instead of its 5-methyl derivative (**II**)*, one of common components in all bleomycin-phleomycin group antibiotics. Lack of a methyl group in a pyrimidine ring was supported by the PMR spectrum of SF-1961.

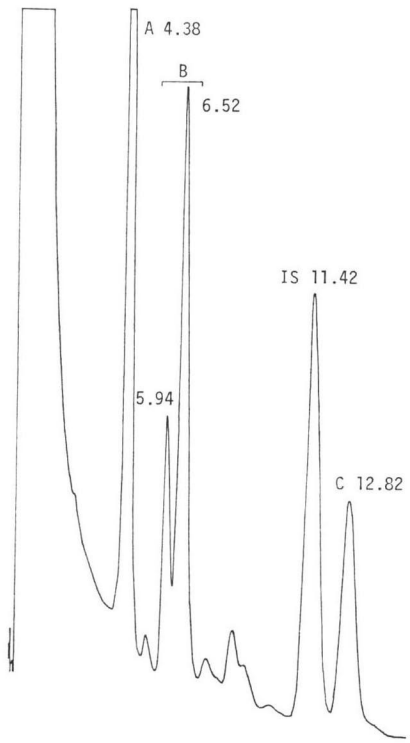
All bleomycin-phleomycin group antibiotics so far reported possessed β-amino-β-(4-amino-6-carboxy-5-methylpyrimidin-2-yl)-propionic acid (**II**)* and demethyl-**II** was only found in the biosynthetic intermediates of bleomycin, isolated from the culture broth of *Streptomyces verticillus*.[9a,b] As reported in the previous paper, SF-1771 also possessed demethyl-**II**. Since the remaining two ninhydrin positive components, which have not yet been identified, moved more slowly than **V** in high voltage electrophoresis, SF-1961 was considered not to possess the terminal amine component.

Neutral sugar components

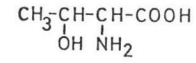
Neutral sugar components of SF-1961 were studied by GC-MS analysis of the methanolyzate (Amberlyst 15 in methanol). Fig. 3 shows the gas chromatogram of TMS-derivatives of the sugar components in SF-1961. As can readily be recognized, SF-1961 contained three neutral sugar components A, B and C. Among them, gulose (B) and 3-O-carbamoyl-mannose (C) were readily identified from their GC-MS spectra. Although the sugar component A has not yet been identified, its molecular formula was considered to be $C_7H_{14}O_6$ from its MS.

* These designations adopted for the components in SF-1961 correspond to those in bleomycin group antibiotics[10a,b].

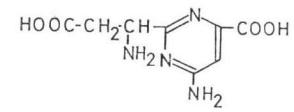
Fig. 3. GLC analysis of neutral sugar components in SF-1961 as their TMS-methyl glycosides. (IS= methyl α-D-glucopyranoside, 0.75% OV-1 on Gaschrom Q 1.2 m, 150°C, He-37 ml/min.)



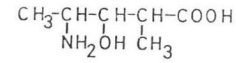
I



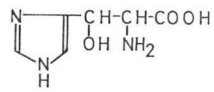
Demethyl-II



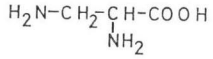
III



IV



V



VI′

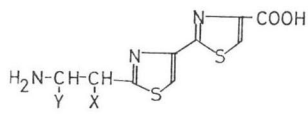
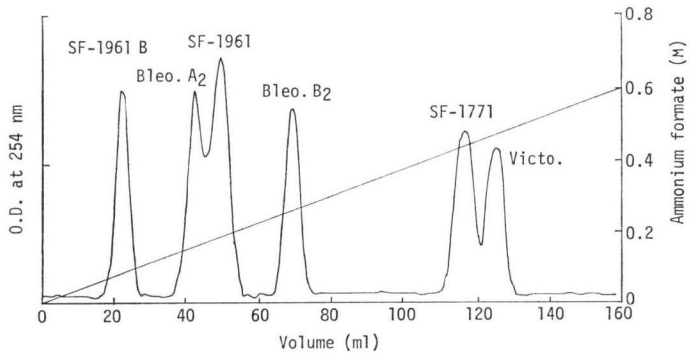


Fig. 4. CM-Sephadex chromatography of SF-1961, SF-1771, bleomycins (Bleo.) and victomycin (Victo.).
Column: CM-Sephadex C-25 (i.d. 8 mm×15 cm)
Elution: 0~0.6 M ammonium formate
Flow rate: 10 ml/hour



The presence of an extra neutral sugar together with presence of demethyl-**II** gave unique features to SF-1961. As bleomycin group antibiotics to which SF-1961 belongs, zorbonomycin[11], victomycin[12], platomycins A and B[13], tallysomycin[6], cleomycin[7], bleomycins[14], YA-56[15] and SF-1771[1] have been reported. However, SF-1961 could be definitely differentiated from other hitherto known bleo-

mycin group antibiotics including bleomycins, YA-56X, tallysomycins because these compounds contained **II** instead of demethyl-**II**. From victomycin and SF-1771, SF-1961 could also be clearly differentiated on CM-Sephadex column chromatography as shown in Fig. 4.

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References

1) OHBA, K.; T. SHOMURA, T. TSURUOKA, S. OMOTO, M. KOJIMA, T. HISAMATSU, S. INOUYE & T. NIIDA: SF-1771, a new antibiotic related to bleomycin. J. Antibiotics 33: 1236~1242, 1980
2) PRIDHAM, T. G. & H. D. TRESNER: BERGEY's Manual of Determinative Bacteriology. 8th edition. pp. 747~829, The Williams and Wilkins Co., Baltimore, U.S.A., 1974
3) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type culture of *Streptomyces*. II. Species descriptions from first study. Intern. J. Syst. Bacteriol. 18: 69~189, 1968
4) TAKITA, T.; Y. MURAOKA, T. NAKATANI, A. FUJII, Y. UMEZAWA, H. NAGANAWA & H. UMEZAWA: Chemistry of bleomycin. XIX. Revised structures of bleomycin and phleomycin. J. Antibiotics 31: 801~804, 1978
5) OHASHI, Y.; H. ABE, S. KAWABE & Y. ITO: The constitutional amine of antibiotic YA-56. Agr. Biol. Chem. 37: 2387~2391, 1973
6) KONISHI, M.; K. SATO, K. NUMATA, T. TSUNO, K. ASAMA, H. TSUKIURA, T. NAITO & H. KAWAGUCHI: Tallysomycin, a new antitumor antibiotic complex related to bleomycin. II. Structure determination of tallysomycins A and B. J. Antibiotics 30: 789~804, 1977
7) MURAOKA, Y.; A. FUJII, H. NAGANAWA, T. TAKITA & H. UMEZAWA: Chemical structure of cleomycin, a new antibiotic related to bleomycin-phleomycin group antibiotics. 55th Annual Meeting of the Agricultural Chemical Society of Japan, Abstract 1P-25, Fukuoka April 1~4, 1980
8) We thank Dr. T. TAKITA of Institute of Microbial Chemistry for generous sample of demethyl-**II**.
9) a) NAKATANI, T.; K. ISHIKAWA, Y. MURAOKA, A. FUJII, H. NAGANAWA, T. TAKITA & H. UMEZAWA: Biosynthesis of bleomycin. Isolation and structure studies of peptide fragments. 53th Annual Meeting of the Agricultural Chemical Society of Japan, Abstract 2P-16, Nagoya, April 2~4, 1978
b) IITAKA, Y.; H. NAKAMURA, T. NAKATANI, Y. MURAOKA, A. FUJII, T. TAKITA & H. UMEZAWA: Chemistry of bleomycin. XX. The X-ray structure determination of P-3A Cu(II)-complex, a biosynthetic intermediates of bleomycin. J. Antibiotics 31: 1070~1072, 1978
10) a) TAKITA, T.; Y. MURAOKA, K. MAEDA & H. UMEZAWA: Chemical studies on bleomycins. I. The acid hydrolysis products of bleomycin A_2. J. Antibiotics 21: 79~80, 1968
b) MURAOKA, Y.; T. TAKITA, K. MAEDA & H. UMEZAWA: Chemistry of bleomycin. IV. The structure of amine component II of bleomycin A_2. J. Antibiotics 23: 252~253, 1970
11) ARGOUDELIS, A. D.; M. E. BERGY & T. R. PYKE: Zorbamycin and related antibiotics. I. Production, isolation and characterization. J. Antibiotics 24: 543~557, 1971
12) TAKASAWA, S.; I. KAWAMOTO, R. OKACHI, M. KOHAKURA, R. YAHASHI & T. NARA: A new antibiotic victomycin(XK-49-1-B-2). II. Isolation, purification and physicochemical and biological properties. J. Antibiotics 28: 366~371, 1975
13) TAKASAWA, S.; I. KAWAMOTO, R. YAHASHI, R. OKACHI, M. YAMAMOTO, T. SATO & T. NARA: Platomycins A and B. II. Physicochemical properties. J. Antibiotics 28: 662~667, 1975
14) UMEZAWA, H.; Y. SUHARA, T. TAKITA & K. MAEDA: Purification of bleomycins. J. Antibiotics, Ser. A 19: 210~215, 1966
15) OHASHI, Y.; H. ABE & Y. ITO: Constitutional amino acids of the antibiotic YA-56. Agr. Biol. Chem. 37: 2277~2282, 1973