

## SETAMYCIN, A NEW ANTIBIOTIC

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A new antibiotic, setamycin, was extracted from the mycelia of a rare actinomycete strain KM-6054. The antibiotic, the molecular formula of which was found to be  $C_{42}H_{81}NO_{12}$  (tentative), is a yellow powder showing activity against some fungi, trichomonads and weakly against Gram-positive bacteria.

In the course of our screening program for new antibiotics from soil microorganisms, a new antibiotic named setamycin has been isolated from the mycelia of an actinomycete strain. The producing organism, strain KM-6054, was isolated from a soil sample collected at Setagaya-ku, Tokyo, in Japan.

This strain is considered not to be classified into any of the known genera of *Actinomycetales* from the morphological characteristics and the diaminopimelic acid constituent of the cell wall.<sup>1)</sup> The substrate mycelia of the strain KM-6054 branch well and fragmentation does not occur. The morphology of the aerial hyphae is classified into the section *Recti-flexibilis*. The spore chains have more than twenty spores with smooth surfaces. In the whole cell hydrolyzate of this strain both LL-diaminopimelic acid and its *meso*-isomer were detected. Since these properties did not coincide with those of any known genus, further studies on taxonomy were carried out to substantiate that this strain belongs to a new genus.<sup>2)</sup> In this paper, fermentation, isolation and characterization of setamycin are presented.

#### Fermentation

The seed culture was prepared in a medium consisting of 1.0% glucose, 2.0% starch, 0.5% yeast extract, 0.5% peptone and 0.4%  $CaCO_3$  in tap water (pH 7.0), and was transferred into a 30-liter fermentor containing 20 liters of the medium composed of 2.0% dextrin, 0.2% glucose, 1.5% soybean meal, 0.3% yeast extract and 0.3%  $CaCO_3$  (pH 7.0). Fermentation was carried out at 27°C for 3 days under aeration (10 liter/minute) and agitation (250 rpm).

The antimicrobial activity of setamycin was assayed with *Piricularia oryzae* by usual paper disc method, where a linear relationship between logarithms of concentrations of the antibiotic and diameters of inhibitory zones were observed in the range of 15~500 mcg/ml. The peak level of the antibiotic production shown in Fig. 1 is approximately 100 mcg/g of wet mycelia.

#### Isolation and Purification

The mycelial cake (1 kg) separated by centrifugation from a cultured broth was extracted with<sup>3)</sup>

Fig. 1. Time course of production of setamycin.

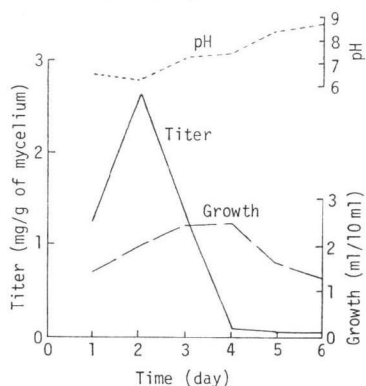
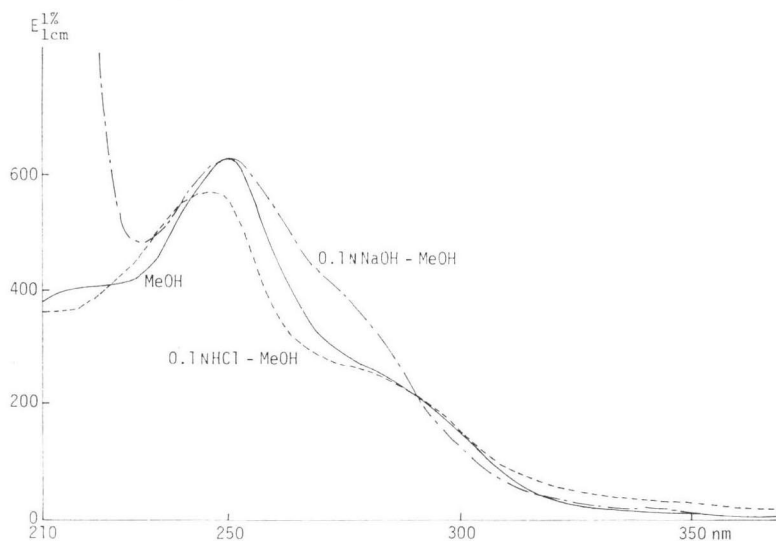


Table 1. Physico-chemical properties of setamycin.

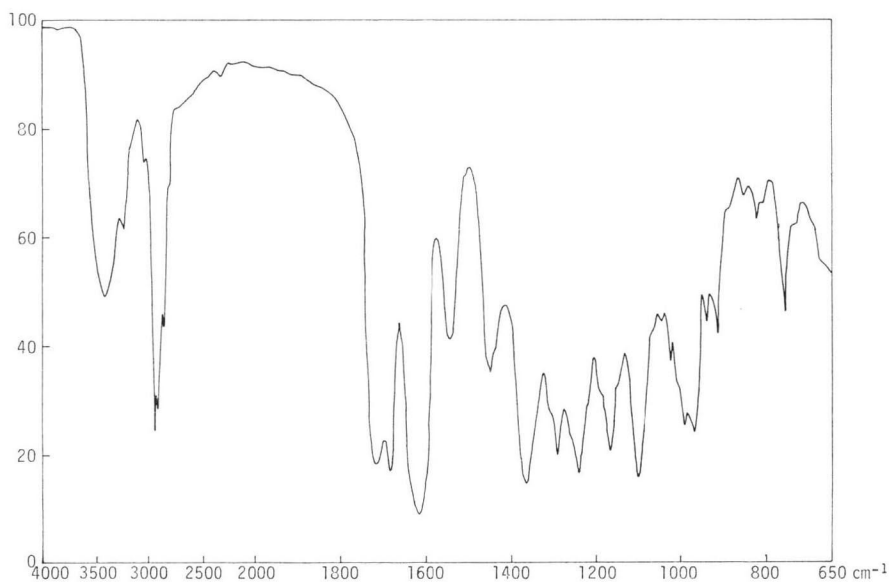
Appearance	yellow powder
Melting point	134~135°C
Optical rotation ( $[\alpha]_D^{28}$ )	+18.3° (c 1, MeOH)
UV $\lambda_{max}$ nm ( $E_{1cm}^{1\%}$ ) (MeOH)	249 (630), 280 (sh 280)
IR (KBr) $cm^{-1}$	3400, 2950, 1720, 1680, 1620, 1540, 1450, 1360, 1240, 1100, 970, 820, 760
Molecular formula	$C_{42}H_{61}NO_{12}$ (771) (tentative)
Elemental analysis: Found	C 65.55; H 7.88; N 1.83; O 24.74
Calcd.	C 65.28; H 7.90; N 1.81; O 24.87
Solubility: Soluble	methanol, ethanol, ethyl acetate, acetone, benzene, chloroform and ether
Insoluble	water and <i>n</i> -hexane
Color reaction: Positive	potassium permanganate, sulfuric acid, aniline phthalate, anisaldehyde- $H_2SO_4$
Negative	ninhydrin, DRAGENDORFF
Rf values on silica gel TLC	
Benzene - acetone (2: 1)	0.25
Ethyl acetate	0.24
Chloroform - methanol (10: 1)	0.48

Fig. 2. Ultraviolet absorption spectra of setamycin.



liters of methanol. The aqueous methanol solution was concentrated under reduced pressure to 700 ml. The antibiotic was extracted five times with 200 ml of ethyl acetate each. After drying over anhydrous sodium sulfate the extract layer was concentrated to a small volume, and washed with *n*-hexane to give 1.6 g of a yellow powder. The crude powder was dissolved in a small volume of acetone to chromatograph in benzene - acetone (10: 1) through a silica gel column. The active fractions were collected and evaporated to yield 820 mg of a yellow powder. The powder (100 mg) dissolved in a small volume of ethyl acetate was further purified by a Sephadex LH-20 column in ethyl acetate to give 80 mg of the pure material of setamycin as yellow powder. This material gave a single spot on silica gel TLC in various developers.

Fig. 3. Infrared absorption spectrum of setamycin (KBr).



#### Physico-chemical Properties

Some properties of setamycin are summarized in Table 1. The antibiotic is a yellow amorphous powder and melts at 134~135°C. It is soluble in methanol, ethanol, ethyl acetate, acetone, chloroform, benzene and ethyl ether, and insoluble in water and *n*-hexane.

The UV spectrum of the antibiotic is as shown in Fig. 2. The peak at 249 nm shifts 5 nm to a shorter wavelength in 0.1 N HCl - MeOH. The IR spectrum is shown in Fig. 3. The number of carbons of the antibiotic are estimated between 39 and 44 by the  $^{13}\text{C}$  NMR spectrum. The molecular formula,  $\text{C}_{42}\text{H}_{81}\text{NO}_{12}$ , is tentatively assigned to setamycin by elementary analysis and  $^{13}\text{C}$  NMR spectrum.

#### Biological Properties

The minimum inhibitory concentration (MIC) of setamycin is given in Table 2. The antibiotic is active against some fungi, trichomonads, and weakly against Gram-positive bacteria. Acute toxicity of setamycin was examined with mice. When 0.6 mg/kg of the antibiotic was given by i.p. injection, all the mice survived, but over 1.25 mg/kg of it killed them all.

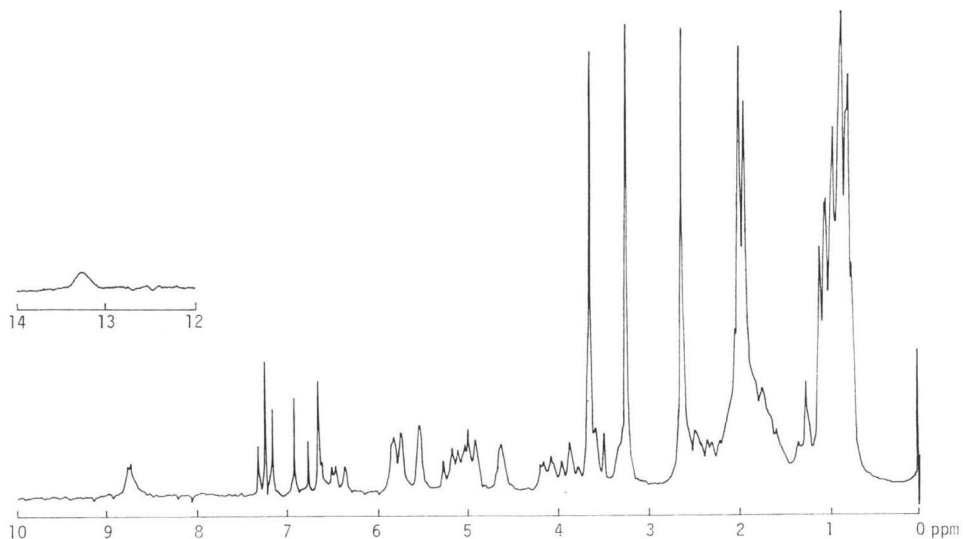
Table 2. Antimicrobial spectra of setamycin.

Test organism	MIC ( $\mu\text{g/ml}$ )	
<i>Bacillus subtilis</i> PCI 219	25	I*
<i>Bacillus cereus</i> IFO 3001	12.5	"
<i>Staphylococcus aureus</i> FDA 209P	25	"
<i>Micrococcus luteus</i> PCI 1001	50	"
<i>Mycobacterium smegmatis</i> ATCC 607	50	"
<i>Escherichia coli</i> NIHJ	>100	"
<i>Klebsiella pneumoniae</i> PCI 602	>100	"
<i>Pseudomonas aeruginosa</i> P-3	>100	"
<i>Xanthomonas oryzae</i>	>100	II
<i>Candida albicans</i>	>100	"
<i>Saccharomyces sake</i>	0.78	"
<i>Piricularia oryzae</i>	6.25	"
<i>Aspergillus niger</i>	100	"
<i>Alternaria kikuchiana</i>	100	"
<i>Mucor racemosus</i> IFO 4851	>100	"
<i>Fusarium oxysporum</i>	>100	"
<i>Botrytis cinerea</i>	100	"
<i>Trichomonas foetus</i>	1.25	III
<i>Trichomonas vaginalis</i> S <sub>1</sub>	0.3	"

\*I: Heart infusion agar.

II: Potato-glucose agar.

III: Trichosel broth (BBL) by broth dilution method.

Fig. 4. 100 MHz  $^1\text{H}$  NMR spectrum of setamycin ( $\text{CDCl}_3$ ).

### Discussion

Setamycin was compared with other known antibiotics having some points of similarity in physical, chemical and biological properties. Among several known antibiotics SF-1540 A<sup>3,4</sup> is the most similar to setamycin. This antibiotic shows UV absorption maxima at 249 and 280 (sh) nm, and SF-1540 A shows almost the same absorption maxima. Melting point, optical rotation, color reaction and analytical value of setamycin are rather similar to those of SF-1540 A. Biological properties of the former are also similar to those of the latter. But the mobility of setamycin on silica gel TLC in benzene - acetone (3: 1) is different from that of an authentic sample of SF-1540 A.

Concerning the IR spectrum, setamycin is also similar to SF-1540 A, but in the  $^1\text{H}$  NMR spectrum the former differs from the latter in the presence of peaks at 3.62 ppm (3H, assignable to  $-\text{OCH}_3$ ), 5.55 ppm (1H, singlet), 6.63 ppm (1H, singlet) and 13.2 ppm (1H, broad singlet) (Fig. 4). From these results setamycin is differentiated from SF-1540 A.

### Acknowledgement

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### References

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