A NEW ENTEROMYCIN GROUP ANTIBIOTIC, YN-0165J-A PRODUCED BY STREPTOMYCES SP.

HARUMITSU IMAI, KEN-ICHI SUZUKI, SHUICHI TAKAMURA, SHIGENOBU KADOTA and MASARU IWANAMI

Bioscience Research Laboratories, Central Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd., 1-1-8, Azusawa, Itabashi-ku, Tokyo, Japan

(Received for publication January 16, 1986)

In the course of our screening for new antibiotics, a Streptomyces strain YN-0165J isolated from a soil sample collected at Omaezaki in Shizuoka Prefecture, Japan, was found to produce a new antibiotic.

The strain YN-0165J is classified in the genus Streptomyces on the basis of the following characteristics: Color of mature sporulated aerial mycerium is in the blue-color series; mature spore chains showing predominantly hooks, loops or incomplete spirals is section Retinaculum-Apertura; spore surface is warty; formation of melanoid pigment is negative. Analysis of whole cell hydrolysate of the strain YN-0165J showed that it contained L-L-diaminopimelic acid and glycine.

The strain was cultured in 500-ml Erlenmeyer flasks containing 60 ml of a medium consisted of potato starch 3.0%, soybean meal 1.5%, yeast extract 0.2%, corn steep liquor 0.5%, MgSO4·7H2O 0.05%, NaCl 0.3% and CoCl2·6H2O 0.001%. The medium was adjusted to pH 7.0 before sterilization. The strain was cultured at 27°C for 72 hours on a rotary shaker. The antibiotic activity was monitored by paper disk assay using Escherichia coli K-12 as a test organism.

The clarified broth (10 liters) was applied to a Diaion HP-20 resin column. After washing

Fig. 1. $^1$H NMR spectrum of YN-0165J-A (100 MHz, DMSO-$d_6$).
with water, the antibiotic was eluted with 50% acetone. The active fractions were collected and concentrated to dryness. The solid residue (10.75 g) was dissolved with CHCl₃ - MeOH, 4:1 and filtered. The filtered solution was concentrated to a small volume, and then chromatographed on silica gel (120 g) eluting with CHCl₃ - MeOH, 9:1. The active fractions were collected and concentrated to afford a white powder. The powder was crystallized from ethyl acetate to give white crystals (267 mg).

The physico-chemical properties of YN-0165J-A are as follows: MP 120-121°C (dec); high resolution CI-MS m/z 204.099 (M+H, C₇H₁₃N₃O₄); color reactions, positive to 0.5% KMnO₄ and ninhydrin, negative to FeCl₃ and Dragendorff; UV λmax nm (ε) 252 (14,840); IR (KBr) cm⁻¹ 3380, 1650, 1590, 1530, 1430, 1240, 1100 and 1000; ¹H NMR (100 MHz, DMSO-d₆) as shown in Fig. 1; ¹³C NMR (D₂O) δ 181.7, 164.4, 144.3, 58.1, 41.6, 35.3 and 27.5; Anal Calcd for C₇H₁₃N₃O₄: C 41.38, H 6.45, N 20.68, Found: C 41.42, H 6.68, N 20.64.

From the results described above, it is considered that antibiotic YN-0165J-A is classified in enteromycin group antibiotics such as enteromycin¹, enteromycin carboxamide², thermycetin³, RP-7080⁴, U-15774⁵, U-22956⁵ and 19A⁶. However, the physico-chemical properties of YN-0165J-A are different from those of the above antibiotics in this group. The analytical and spectroscopic data of YN-0165J-A indicated above, suggested that the structure was 4-[2-(N-oxidemethoxyimino)acetamidobutyramide, as shown in Fig. 2. Consequently, YN-0165J-A is considered to be a new antibiotic. The antimicrobial activity of YN-0165J-A is shown in Table 1.

Table 1. Antimicrobial spectrum of YN-0165J-A.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em> ATCC 6633</td>
<td>25</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em> ATCC 9341</td>
<td>25</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> Smith</td>
<td>50</td>
</tr>
<tr>
<td>Escherichia coli NIHJ</td>
<td>12.5</td>
</tr>
<tr>
<td>Morganella morganii IID 602</td>
<td>25</td>
</tr>
<tr>
<td>Enterobacter cloacae 963</td>
<td>100</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa NCTC 10490</td>
<td>100</td>
</tr>
</tbody>
</table>

The MIC were determined by a serial agar dilution method with Mueller-Hinton medium.

Inoculum size: 10⁶ cfu/ml.

Acknowledgments

The authors are grateful to the staffs of The Chemotherapy Research Department for measurement of antimicrobial activity and the staffs of The Physico-analysis Center for measurement of spectra and useful suggestions.

References