ISOLATION AND CHARACTERIZATION OF SARUBICIN A,  
A NEW ANTIBIOTIC  

G. REINHARDT, G. BRADLER, K. ECKARDT,* D. TRESSELT and W. IHN  

Akademie der Wissenschaften der DDR, Forschungszentrum für Molekularbiologie und Medizin, Zentralinstitut für Mikrobiologie und Experimentelle Therapie, DDR-69 Jena, Beutenbergstraße 11, DDR  

(Received for publication May 30, 1980)  

The new antibiotic sarubicin A [red crystals, mp. 194–195°C, C₁₃H₁₄N₂O₆ (I)] was isolated from fermentations of a *Streptomyces* strain. The compound is moderately active *in vitro* against *Micrococcus luteus*.  

An antibiotic, designated sarubicin A** was isolated from the culture filtrate of *Streptomyces* strain JA 2861. On the basis of taxonomic studies the producing strain was found to be related to *Streptomyces violaceoruber*. The present communication describes the isolation of crystalline sarubicin A and its chemical and biological characteristics.  

**Experimental**  

**Fermentation procedure for production of sarubicin complex**  

*Streptomyces* strain JA 2861, resulted from a screening program and stored in the lyophilized state was used for fermentation. For short-term maintenance the culture was grown to sporulation for 10–12 days at 28°C on agar slants consisting of 0.3 % saccharose, 1.5 % dextrin, 0.01 % carbamide, 0.1 % yeast extract, 0.5 % Bacto Pepton “Difco”, 0.05 % NaCl, 0.05 % KH₂PO₄, 0.001 % FeSO₄·7H₂O, 3.0 % agar-agar, pH 6.8–7.0, and then kept at 4°C.  

The following seed stage medium was found to be useful to produce a vegetative inoculum: 1.5 % glucose, 1.5 % soya bean meal, 0.5 % NaCl, 0.1 % CaCO₃, 0.03 % KH₂PO₄, pH 6.8. A spore suspension was used to inoculate this medium and was incubated for 48 hours at 28°C on a rotary shaker. The vegetative mycelium of the seed stage was used to inoculate the fermentation medium consisting of 2 % glucose, 1 % soya bean meal, 0.5 % NaCl, 0.3 % CaCO₃, 0.5 % solids of corn steep liquor, pH 6.8, and incubated for 3–4 days at 28°C.  

Shake flask seed and final fermentation were carried out on the laboratory scale in 500-ml cylindrical culture flasks containing 80 ml of medium and incubated at 28°C on a rotary shaker at 180 rpm and with a throw of 45 mm. Pilot scale fermentations were carried out in glass and stainless-steel fully baffled stirred fermenters, respectively. A seed stage using the medium described was inoculated with a spore suspension and incubated at 28°C for 48 hours. Final fermentations were carried out in fermenters containing 20 or 450 liters of the described medium inoculated with 5 % of vegetative seed growth and cultured at 28°C for 3–4 days. Seed and final fermentations were carried out with an agitation rate of 300 rpm and an air flow rate of 1.0 (vol/vol)/min.  

The production of sarubicin complex was followed by a hole plate diffusion method with *Micrococcus luteus* (*Sarcina lutea SG 125A*) as the test organism.  

**Isolation of the antibiotic**  

The culture filtrate of harvested mash was extracted at pH 4.0 with 0.3 volume of *n*-butanol. After  

**In a preliminary short communication** this antibiotic was named sarcinamycin A on its specific activity against *Sarcina lutea*. The term of the test organism has been revised to *Micrococcus luteus*. To avoid misunderstandings we felt it necessary to introduce the new name sarubicin A for the antibiotic.
evaporation of the extract the crude concentrate was chromatographed on aluminium oxide (activity of aluminium oxide was reduced by addition of 10% of water). The material was eluted with chloroform followed by further elution with butanol. A red active substance crystallized from the main red fraction. The compound was then chromatographed on a silica gel column (KH₂PO₄-buffered silica gel, 30 × 3 cm). Elution was with ethyl acetate. The main red band was accompanied with two minor yellow bands. The red fractions of several columns were combined and concentrated to a small volume. After cooling crystalline sarubicin A was obtained. Analytically pure substance was prepared by recrystallization from ethyl acetate.

Analytical procedure
Antibiotic activity (MIC) was determined by the agar plate diffusion method with different test organisms. Purified samples were dissolved in methanol and diluted with water.

Results
Physical and Chemical Properties
Sarubicin A is a red crystalline antibiotic which is soluble in lower alcohols, acetone, chloroform, and other common organic solvents. It is less soluble in water. In concentrated H₂SO₄ the antibiotic dissolves with yellow colour. Crystals of sarubicin A melt at 194–195°C. Elementary analysis gave C, 52.91; H, 4.64; N, 9.17%. C₁₃H₁₁N₃O₆ requires C, 53.06; H, 4.76; N, 9.50%. The ultraviolet and visible absorption spectrum of sarubicin A in chloroform contains maxima at 262, (286), and 498 nm (logs 4.10, (3.88), 3.20) (Fig. 1). The infrared spectrum (Fig. 2) in KBr had characteristic absorptions at 1605 and 1645 cm⁻¹.

Structure I (Fig. 3) has been deduced from detailed studies of sarubicin A and its monoacetate.³

Biological Properties
The agar plate diffusion method was used to determine the antibacterial activity. Sarubicin A is active against Micrococcus luteus but is not or only slightly active against other strains of bacteria tested (Table 1). It did not inhibit the growth of yeasts and fungi tested.

Discussion
The Streptomyces strain JA 2861 was found to produce a mixture of new antibiotically active pigments. The main component designated as sarubicin A was isolated as red crystals. Structural elucidation studies of this compound, details of which will be published elsewhere³, have indicated that sarubicin A has structure I. On the basis of these results and its physico-chemical as well as biological characteristics sarubicin A was found to be a new antibiotic. Its chemical structure shows some interesting relations to the structures of granaticin and granaticin B¹,², dihydrogranaticin⁵, and granaticinic acid⁶.
Fig. 2. IR spectrum of sarubicin A in KBr.

Table 1. Antimicrobial activity of sarubicin A (agar plate diffusion test).

<table>
<thead>
<tr>
<th>Test organism</th>
<th>MIC μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis ATCC 6633</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Bacillus globifer OH 11</td>
<td>100</td>
</tr>
<tr>
<td>Bacillus mycoides SG 756</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Staphylococcus aureus SG 511</td>
<td>100</td>
</tr>
<tr>
<td>Micrococcus luteus (Sarcina lutea SG 125A)</td>
<td>15</td>
</tr>
<tr>
<td>Escherichia coli mutable SG 458</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Escherichia coli C 600</td>
<td>50</td>
</tr>
<tr>
<td>Proteus vulgaris Ox 19 SG 2</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Klebsiella aerogenes SG 117</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Alcaligenes faecalis ATCC 8750</td>
<td>25</td>
</tr>
<tr>
<td>Comamonas terrigena ATCC 8461</td>
<td>50</td>
</tr>
<tr>
<td>Mycobacterium phlei SG 346</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

In concentrations of 100 μg/ml sarubicin A did not inhibit the growth of yeasts and fungi tested.

Fig. 3. Sarubicin A.

Fig. 4. Mass spectrum of sarubicin A.
References


