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

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The new antibiotic sarubicin A [red crystals, mp. 194~195°C, $C_{13}H_{14}N_2O_6$ (**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% KH_2PO_4, 0.001% $FeSO_4 \cdot 7H_2O$, 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% $CaCO_3$, 0.03% KH_2PO_4, 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% $CaCO_3$, 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[1] 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 (KH_2PO_4-buffered silica gel[2], 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

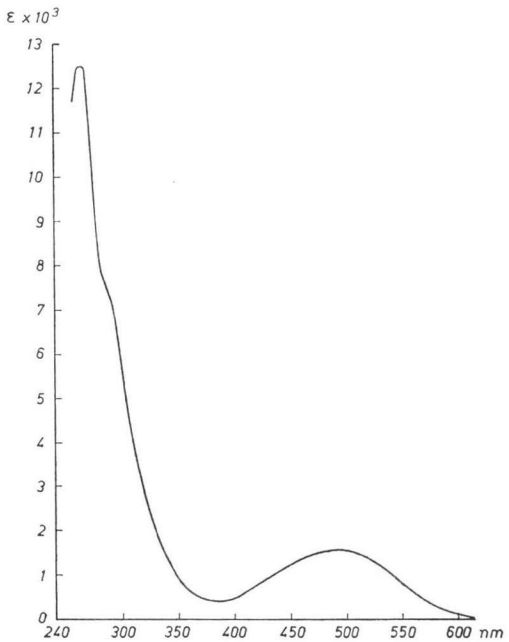
Antibiotic activity (MIC) was determined by the agar plate diffusion method with different test organisms. Purified samples were dissolved in methanol and diluted with water.

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 H_2SO_4 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%. $C_{13}H_{14}N_2O_6$ 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 (log ε 4.10, (3.88), 3.20) (Fig. 1). The infrared spectrum (Fig. 2) in KBr had characteristic absorptions at 1605 and 1645 cm^{-1}.

Fig. 1. UV spectrum of sarubicin A in chloroform.



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 elsewhere[3], 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 B[4,5], dihydrogranaticin[6], and granaticinic acid[7].

Fig. 2. IR spectrum of sarubicin A in KBr.

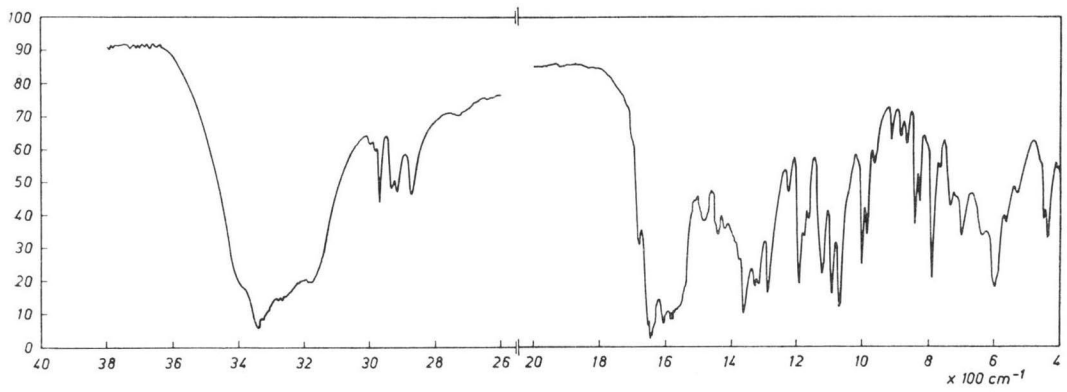
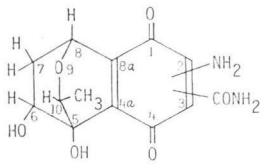


Table 1. Antimicrobial activity of sarubicin A (agar plate diffusion test).

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

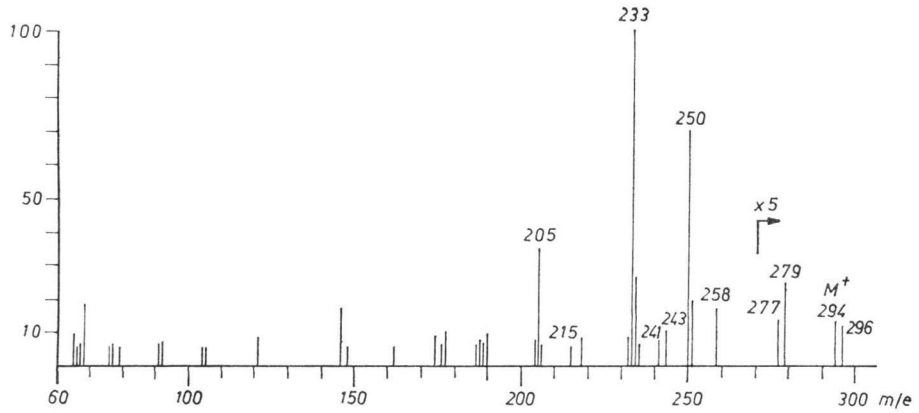
In concentrations of 100 μg/ml sarubicin A did not inhibit the growth of yeasts and fungi tested.

Fig. 3. Sarubicin A.



I

Fig. 4. Mass spectrum of sarubicin A.



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