THE STRIATINS—NEW ANTIBIOTICS FROM THE BASIDIOMYCETE *CYATHUS STRIATUS* (HUDS. ex PERS.) WILLD.

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Three crystalline antibiotics which we named striatins A, B, and C were isolated from the mycelium of the basidiomycete *Cyathus striatus* strain No. 12. The striatins are highly active against fungi imperfecti and a variety of Gram-positive bacteria, as well as against some Gram-negative bacteria. The molecular formulas as determined by mass spectrometry are $C_{27}H_{36}O_7$ for striatin A, $C_{27}H_{36}O_8$ for striatin B, and $C_{25}H_{34}O_7$ for striatin C.

In the course of our screening for new metabolites from basidiomycetes we found that a strain of *Cyathus striatus* which was isolated by Mrs. L. KISIMOVA-HOROVITZ produces several antibiotics when grown in submerged culture. From the mycelium of this strain we isolated three crystalline antibiotics which we have named striatins A, B, and C. These antibiotics do not belong to the cyathin group of antibiotics[1,2], which were isolated from *Cyathus helenae* by ALLBUTT *et al.*[3] The cyathins are oxygenated C_{20} compounds belonging to a new group of diterpenoids. Their physico-chemical properties (UV-, IR-, and mass-spectra) as well as their biological properties are completely different from those exhibited by the striatins. In the following paper we wish to report the fermentative production, the isolation, and the biological and chemical characterization of the striatins.

Fermentation

Cyathus striatus No. 12 was maintained on agar slants of a yeast extract-malt extract (YM) medium (4 g yeast extract, 4 g glucose, 10 g malt-extract per liter). For submerged cultivation, a 150-ml of YM medium was inoculated with mycelium from one agar slant and incubated for 7 days on a rotary shaker. This culture was used to inoculate 10 liters of the same medium in a New Brunswick fermentation apparatus. Two ml of polyol antifoam were added initially, and the mycelia were grown at 22°C with mechanical stirring (200 rpm) and an aeration rate of 3 liters air/min.

Isolation

The cells from the 10-liter culture grown for 6~8 days were collected on a Buchner funnel, washed with water, and extracted with several batches of methanol - acetone (2: 1). The combined extracts were evaporated to dryness, the residue dissolved in methanol, loaded on a column (2.5×74 cm) with

Sephadex LH–20, and eluted with the same solvent. The fractions containing the striatins (V_e/V_t 0.73～0.90) were pooled, concentrated to 1/10 of the original volume, and kept at 4°C. After 2～3 days the crystals of striatin A (400 mg) were removed, and the mother liquid evaporated. The residue containing the striatins B and C were dissolved in chloroform and applied to a column with silica gel (Mallinckrodt). Striatins B and C were eluted with chloroform - ethanol (99: 1) and chloroform - ethanol - methanol (97: 1: 2) and crystallized from chloroform (striatin B) and ethanol (striatin C). Yield: 700 mg striatin B and 300 mg striatin C.

Physico-chemical Properties

The striatins obtained are colorless, crystalline antibiotics with melting points of 144～145°C (striatin A), 143～144°C (striatin B), and 144～145°C (striatin C). They are soluble in methanol, ethanol, ethyl acetate and chloroform, and are poorly soluble in water and cyclohexane. The UV-spectra of the striatins in ethanol show no absorption maxima up to 220 nm. The chromatographic behavior of the striatins on thin-layer chromatography is reported in Table 1. The antibiotics give positive reactions with $KMnO_4$ and a modified KAGI-MIESCHER reagent[4]. Upon standing in solution the striatins undergo rapid transformation into several new compounds, of which some show antibiotic activity. Since these transformations do also occur in the solvents used for NMR-spectroscopy, the interpretation of the NMR-spectra needs further investigation. The IR-spectra of the striatins A, B, and C are shown in Figs. 1～3. Fig. 4 shows the mass spectrum of striatin C. High resolution of the molecular ions *m/e* 472, 488, and 446 yielded the formulas $C_{27}H_{36}O_7$, $C_{27}H_{36}O_8$, and $C_{25}H_{34}O_7$ for the striatins A, B, and C respectively. All compounds show prominent M–CH_3 peaks, and a characteristic fragment *m/e* 203 ($C_{15}H_{23}$).

Table 1. Chromatographic behavior of striatins A, B and C.

TLC was performed on Merck silica gel plates and the spots detected by spraying with $KMnO_4$ in water or by bioautography on agar plates seeded with *Bacillus brevis* ATCC 9999.

Solvent system	Rf		
	A	B	C
Benzene - methanol - ethyl acetate 17: 2: 1	0.51	0.39	0.24
Cyclohexane - ethylacetate 1: 1	0.38	0.30	0.16
Benzene - acetone - acetic acid 70: 30: 1	0.55	0.47	0.26

Biological Properties

Table 2 shows the antimicrobial spectra of the striatins in the serial dilution test and the agar plate diffusion test. They are active against fungi imperfecti, aerobic and anaerobic Gram-positive bacteria, and some Gram-negative bacteria. Yeasts are not affected by concentrations up to 20 μg/ml; due to the limited solubility of the striatins in water no higher concentrations could be used in the serial dilution test. Yeasts and fungi, *Arthrobacter citreus, Sarcina lutea* and *Streptomyces viridochromogenes* were grown on YM medium, *Clostridium pasteurianum* on Merck RCM medium 5411, and the other bacteria on nutrient broth (Difco).

Effect on Protein, RNA, and DNA Synthesis

The effect of the striatins on macromolecular synthesis was tested by adding the appropriate precursors to exponentially growing cells of *Bacillus brevis* in nutrient broth. The radioactively labelled

Fig. 1. IR-spectrum of striatin A in KBr.

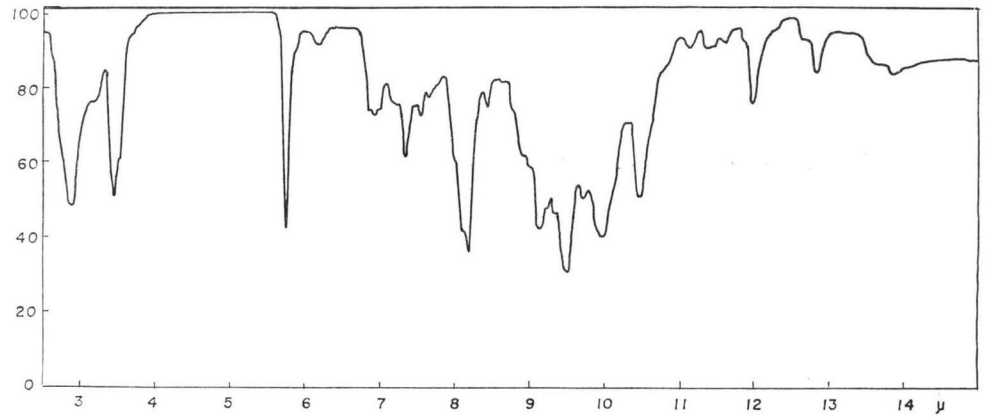


Fig. 2. IR-spectrum of striatin B in KBr.

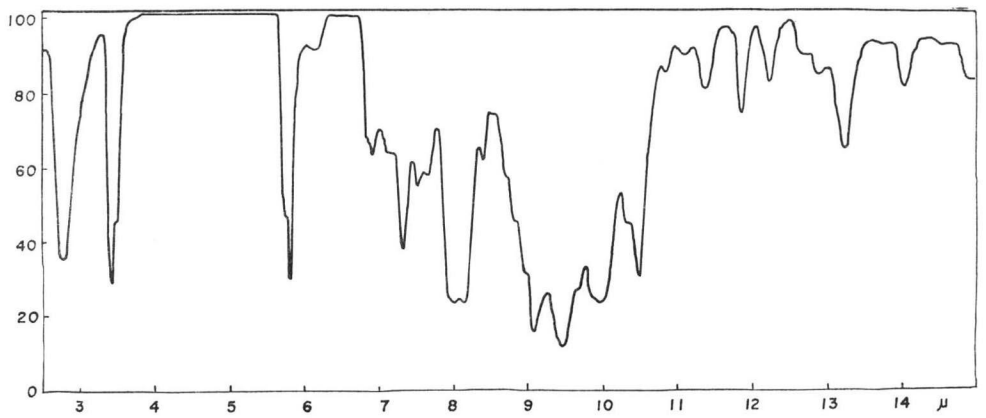


Fig. 3. IR spectrum of striatin C in KBr.

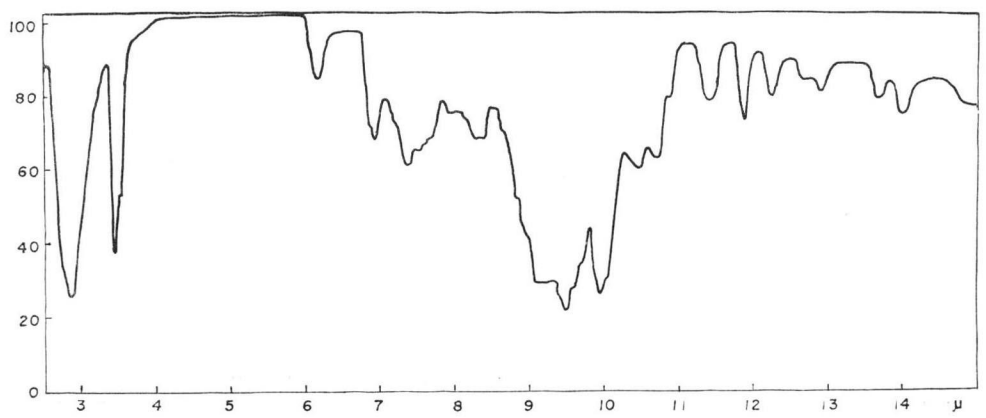


Fig. 4. Mass spectrum of striatin C.
Mass spectra were determined on an A.E.I. MS 50 mass spectrometer (70 eV, direct insertion, 150°).

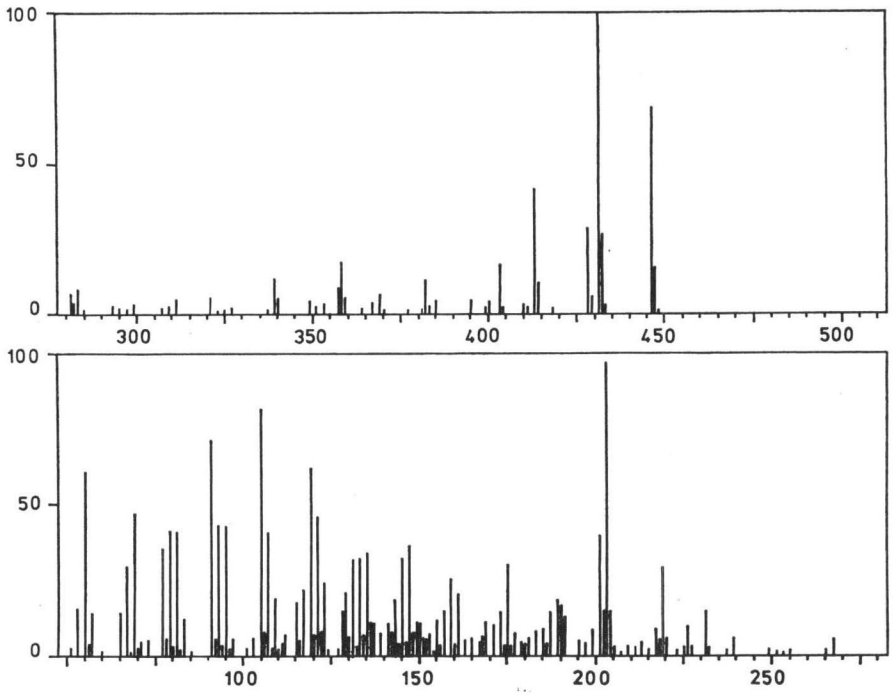


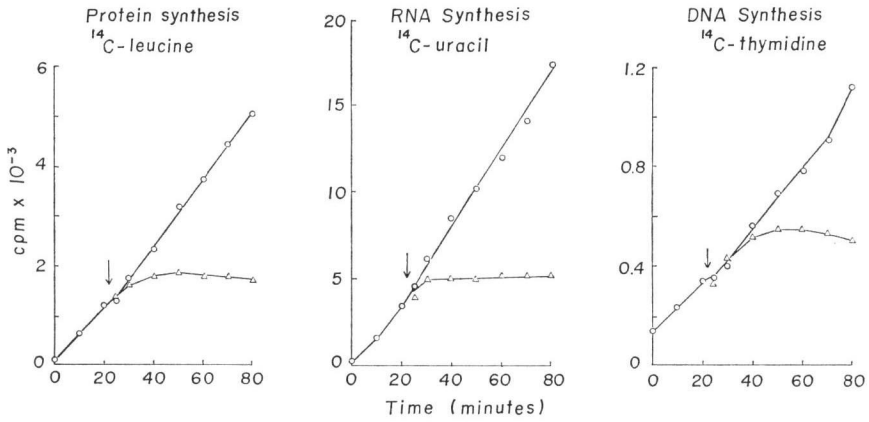
Table 2. Antimicrobial spectra of the striatins A, B and C

	Test organism	MIC (μg/ml)		
		Striatin A	Striatin B	Striatin C
Serial dilution test	*Arthrobacter citreus*	2~5	5~10	2~5
	Bacillus brevis	6	6	6
	Bacillus subtilis	0.2~2	2~20	0.2~2
	Escherichia coli K12	>20	>20	>20
	Leuconostoc mesenteroides	10~15	15~20	15~20
	Mycobacterium phlei	2~20	2~20	2~20
	Nocardia brasiliensis	2~20	2~20	2~20
	Proteus vulgaris	0.2~2	0.2~2	0.2~2
	Pseudomonas fluorescens	>20	>20	>20
	Sarcina lutea	10~15	10~15	10~15
	Staphylococcus aureus	2~5	5~10	2~5
	Streptomyces viridochromogenes	2~20	2~20	2~20
	Saccharomyces cerevisiae	>20	>20	>20
	Rhodotorula rubra	>20	>20	>20
Diameter inhibition zone (mm) with 20/100 μg per paper disc**	*Clostridium pasteurianum*	12/nt*	9/nt	10/nt
	Aspergillus panamensis	14/17	—/14	10/22
	Penicillium notatum	13/16	14/21	17/24
	Fusarium cubense	10/20	8/12	7/12

* nt: not tested.

** The figures before the slash refer to the size of the inhibition zone with discs (diameter 6 mm) receiving 20 μg striatin A, B, or C; the figure after the slash refers to the inhibition zone with 100 μg antibiotic per disc.

Fig. 5. The effect of striatin A on macromolecular synthesis.



precursors (0.08 μCi=0.2 μg L-(1-^{14}C)-leucine, 0.08 μCi=0.3 μg (2-^{14}C)-thymidine, 0.08 μCi=30 μg (2-^{14}C)-uracil per ml culture) were added at zero time, the antibiotic (12.5 μg/ml) was added 23 minutes later and the incorporation (cpm/ml culture) into the 5% trichloroacetic acid insoluble fraction of cells was determined at suitable time intervals before and after the addition of the striatin and also in a control culture containing no antibiotic. The acid-insoluble material was collected on membrane filters, and the radio activity estimated by liquid scintillation counting. As shown in Fig. 5, striatin A inhibits protein and RNA synthesis after 5 minutes and DNA synthesis 15 minutes after addition of the antibiotic. With striatin C, which was tested the same way, similar results were obtained. However, the results of these experiments do not allow conclusions about the primary target of the antibiotics.

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