Myroridin, a New Peptide Antibiotic from *Myrothecium* SP. 285F

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KONDO, S. *Myroridin, a New Peptide Antibiotic from Myrothecium SP. 285F* Tohoku J. exp. Med., 1977, 122 (4), 403-404 — Myroridin, a new basic peptide antibiotic was isolated from culture filtrate of a strain of *Myrothecium*, No. 285F. The present paper describes the production, isolation and characterization of this antibiotic. — *Myrothecium*; myroridin; peptide antibiotic

Myroridin was produced by fermenting a strain of *Myrothecium*, No. 285F, at 24~28°C for 14~16 days in a jar fermentor. The taxonomic characteristics of the strain No. 285F are very similar to those of *Myrothecium roridum*. The fermentation medium contained 3.0% glucose, 1.0% polypeptone, 0.2% NaNO₃, 0.1% KH₂PO₄, 0.05% KCl, 0.05% MgSO₄·7H₂O, 0.001% FeSO₄·7H₂O. To remove cationic impurities, the culture filtrate was applied to the IRC-50 (H-type) column. The antibiotic was eluted with 1 N HCl. After concentration of the eluate, the aqueous solution of the antibiotic was precipitated with acetone-ethanol (4:1) and then the precipitate was dissolved in methanol. Methanol was evaporated and thus crude powder was obtained. When the crude powder was dissolved in methanol, washed with acetone, dissolved in water, and applied to a column of Al₂O₃, or Biogel P-2 or CM Sepadex, two major peaks which were named Fraction I and Fraction II were obtained.

The optical rotation of Fraction I was $[\alpha]_D^{22} + 0.2$ (c=1%, H₂O). The elementary analysis gave the following composition: C 40.19; H 6.90; N 17.27; Cl 14.58 (%). The molecular weights estimated by means of gel filtration, freezing point and steam distillation were 1300, 1300 and 1200, respectively. It melts at 192~198°C with decomposition. The ultraviolet absorption spectrum in methanol exhibited the maximum absorption at 278 nm ($E_{1\text{cm}}^{1\%} = 1175$). Fig. 1 shows the IR spectrum in KBr. Hydrolysis in 6 N HCl at 110°C for 24 hr in a sealed tube and subsequent amino acid analysis revealed the predominant presence of arginine, threonine, hydroxyarginine, tyrosine, ornithine and lysine in Fraction I.

The optical rotation of Fraction II was $[\alpha]_D^{22} + 0.6$ (c=1%, H₂O). The elementary

Fig. 1. Infrared spectra of Fraction I (A) and Fraction II (B).

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analysis gave the following composition: C; 37.78; H 6.77; N 19.53; Cl 13.29%. The molecular weights determined by gel filtration, freezing point and steam distillation were 1200, 1220 and 1200, respectively. It melts at 197~203°C with decomposition. The ultraviolet absorption spectrum in methanol exhibited the maximum absorption at 278 nm ($E_{1\text{cm}}^{1\%}=1175$). The infrared spectrum in KBr tablet is shown in Fig. 1(B). Hydrolysis in 6 N HCl at 110°C for 24 hr in a sealed tube and subsequent amino acid analysis revealed the predominant presence of arginine, threonine, hydroxyarginine, tyrosine and ornithine in Fraction II. The behaviors of Fraction 1 or 2 towards chemical test in color reaction were exactly the same. Namely, they gave positive reactions to Ninhydrin, Xanthoprotein, Millon, Sakaguchi and Pauli, but negative to Molish, Tollens and Sodium nitroprusside. Also in precipitation reaction they gave positive formations to phosphotungstic acid, picric acid, flavianic acid, pentachlorophenol and benzaldehyde. They are soluble in water and methanol, and insoluble in ethanol, acetone, ether, benzene, chloroform, n-hexane, cyclohexane and petroleum ether. The nitrogen content, the behavior in color reaction and the absorption bands of amide linkages in the infrared absorption spectrum indicate the peptide nature of Fraction I and Fraction II.

TABLE 1. *Antimicrobial spectrum of Fraction I and Fraction II*

Fr. I 0.2, Fr. II 0.4: <i>Candida albicans</i> , <i>Candida tropicalis</i> , <i>Candida pseudotropicalis</i> , <i>Candida utilis</i> , <i>Candida guilliermondai</i> , <i>Candida krusei</i> , <i>Saccharomyces cerevisiae</i> Br-60, <i>Saccharomyces rouxii</i> Boutroux, <i>Zygosaccharomyces salsa</i> , <i>Willia anomala</i> , <i>Hansenula anomala</i> , <i>Torulaspora delbruecki</i> , <i>Rhodotorula rubra</i> , <i>Mycotorula japonica</i> , <i>Debaryomyces hansenii</i> , <i>Pullularia pululans</i>
Fr. I 20, Fr. II 40: <i>Proteus</i> OX-19
Fr. I >100, Fr. II >100: <i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> 209-P, <i>Escherichia coli</i> , <i>Shigella sonnei</i> , <i>Sarcina lutea</i> Hata, <i>Bacillus mycoides</i>
Fr. I >1000, Fr. II >1000: <i>Mycobacterium timothee</i> , <i>Mycobacterium smegmatis</i> , <i>Mycobacterium H₃₇RV</i> , <i>Trichophyton asteroides</i> , <i>Trichophyton rubrum</i> , <i>Trichomonas vaginalis</i>

As shown in Table 1, the antibiotic was effective against yeasts, but inactive against other Gram-positive bacteria, Gram-negative bacteria and Mycobacterium.

Acute toxicities of Fraction I in mice were 144~172.8 mg/Kg, 360~432 mg/Kg, 320~500 mg/Kg and >6000 mg/Kg upon intravenous, intraperitoneal, subcutaneous and oral administration, respectively. Its subacute toxicity in Douryu rat was 200~400 mg/Kg intraperitoneally. Fraction II exhibited almost the same toxicity as Fraction I.

The further characterization and the clarification of the mechanism of action of myroridin are in progress.

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