NOTES

CRYSTALLIZATION AND ANTIFUNGAL ACTIVITY OF PRIMYCIN

JOSEPH V. URI and PAUL ACTOR

Research & Development Division
Smith Kline & French Laboratories
Philadelphia, Pennsylvania 19101, U.S.A.

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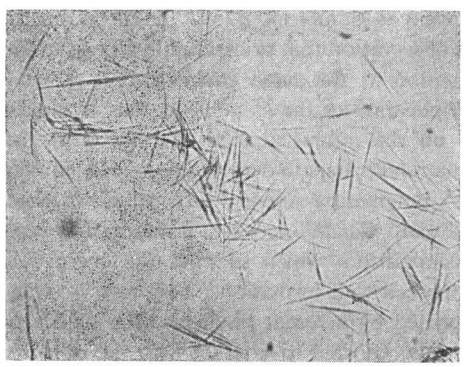
Primycin was first described in 1954 as an amorphous yellowish powder produced by *Streptomyces primycini*[1]. It was reported to be a highly active antibiotic against Gram-positive bacteria as well as pathogenic and nonpathogenic mycobacteria[2]. Due to its toxicity in experimental animals, primycin is limited to topical use. Later it was found to inhibit the growth of *Euglena gracilis* and *Astasia longa* by interacting with the single stranded forms of nucleic acids[3]. Primycin is also produced by a newly isolated strain of *Micromonospora galeriensis*[4]. The chemical structure of primycin was elucidated and characterized. It is the largest non-polyene macrolide ring reported to date, and unique in that this highly complex molecule contains both a guanidine and an arabinose unit[5,6,7].

Recently, we were able to obtain primycin in crystalline form and found it to possess antifungal activities in addition to its previously reported antimicrobial spectrum. These new results with an old antibiotic are discussed in this paper.

The process of crystallization of primycin is, in some respects, unique. Starting with the highly purified light yellowish amorphous powder (mistakenly referred to as a microcrystalline substance) it was suspended in any of the following solvents: dimethylformamide, dimethylsulfoxide or dimethylacetamide. After addition of 10~15% methanol, the suspension was kept at 37°C for 5~10 days until all the amorphous particles gradually transformed into needle-form crystals (Fig. 1). After filtering and washing with methanol and drying with acetone, snow-white crystalline material was obtained. The contaminating microbiologically inactive yellow pigment remained in the mother liquor.

Fig. 1. Primycin crystals from dimethylformamide/dimethylsulfoxide/methanol.



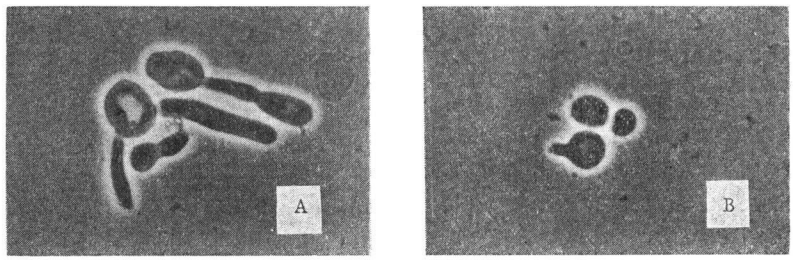
In comparative antimicrobial assay against several Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus faecalis*) and one strain of *Mycobacterium phlei* no noticeable difference was found in the activity of the amorphous and the crystalline primycin preparations. This indicated that the effect of the impurity(s) on biological activity is probably minimal, however, its presence may prevent the crystallization of the active antibiotic. The antibacterial activity of the crystalline primycin is presented in Table 1. The minimal inhibitory concentrations (MIC) were obtained in the pepton-glucose-buffered broth (PGB) which is composed of 0.5% peptone,

Table 1. Antibacterial activity of crystalline primycin against Gram-positive cocci and acid-fast bacterium in peptone-glucose-buffered broth.

Strain	MIC (μg/ml)
Staphylococcus aureus ATCC 25923	>2.0
Staphylococcus aureus 674	0.25
Staphylococcus aureus 209P	0.25
Staphylococcus aureus 303	0.50
Staphylococcus aureus 873	0.25
Staphylococcus aureus 127 (670)*	0.25
Staphylococcus aureus 910R*	0.50
Staphylococcus aureus 660*	0.50
Staphylococcus aureus 671*	0.25
Staphylococcus aureus 664*	0.50
Streptococcus faecalis 34358	0.50
*Mycobacterium phlei**	0.06

* β-Lactamase-producing strains

Fig. 2. Germ tube formation (A) and its partial inhibition (B) by killing concentration (2 μg/ml) of primycin in peptone-glucose-buffered broth (*Candida albicans* #759).



0.1% glucose and 10% McILVAINE's buffer (pH 7.4)[8,9,10]. All the *Staphylococcus* strains (β-lactamase producers and non-producers) and the *Streptococcus faecalis* strain were uniformly inhibited by 0.25~0.5 μg/ml of crystalline primycin with the exception of the ATCC 25923 strain that, for unknown reason, was not inhibited by 2 μg/ml (the highest concentration used). The one strain of *Mycobacterium phlei* tested showed the same high degree of sensitivity to primycin as was described previously[2]. The crystalline primycin, like its amorphous predecessor, possesses no activity against Gram-negative bacilli. The inhibition zones, using the disc agar-diffusion method, were concentration related. *Bacillus subtilis* ATCC 6633 was included to the disc-diffusion assay and showed the highest degree of sensitivity to crystalline primycin (inhibition zone with 0.06 μg/ml).

Studying the antimicrobial spectrum of the crystalline primycin, we have found that it has antifungal activity against a number of *Candida* strains as well as *Trichophyton mentagrophytes* #1258. The data of this study are presented in Table 2. The MIC determinations were carried out in the peptone-glucose-buffered medium (pH 7.4), instead of the SABOURAUD's glucose broth (pH 5.5~6.0) because the nature of primycin is such that it tends to have diminished activity in acidic environment. The MIC's against the *Candida* strains varied between 2 and 10 μg/ml. The *T. mentagrophytes* strain was inhibited by 1.0 μg/ml. The minimum candidacidal concentration for primycin against *C. albicans* #759 was equal to the MIC (2 μg/ml). Furthermore, it was observed that the same concentration blocks the germ tube formation for this strain in the peptone-glucose-buffered broth. This medium was found to support germ tube formation (2 hours, 30°C). At the inhibitory concentration, only rudimentary germ tubes were formed which remained stunted and ultimately the cells died (Fig. 2). Of the non-*albicans* strains, only *C. stellatoidea* forms germ tubes in this medium and this strain has the same degree of sensitivity to primycin as *C. albicans* #759. Based on our present observation that primycin inhibits germ tube formation at the same concentration at which multiplication of the *C. albicans* cells is blocked and on the published data that the germ tube formation appears to depend upon mitochondrial DNA polymerase activity[11], the target organelle for the anti-*Candida* action of primycin may be the mitochondrium which supplies the energy also for the cell wall formation. This view is further supported by a recent publication in which primycin is reported to interact with the energized

Table 2. Antifungal activity of crystalline primycin in peptone-glucose-buffered broth.

Strain	MIC (μg/ml)
Candida parapsilosis 2242	10.0
Candida guillermondii 2243	5.0
Candida krusei 2244	5.0
Candida stellatoidea 2245	2.0
Candida utilis (995) 2253	5.0
Candida albicans 2217	4.0
Candida albicans 2218	5.0
Candida albicans 2219	5.0
Candida albicans 2220	5.0
Candida albicans 2221	2.0
Candida albicans 2222	10.0
Candida albicans 2223	4.0
Candida albicans 2224	5.0
Candida albicans 311	5.0
Candida albicans 759	2.0
Trichophyton mentagrophytes 1258	1.0

and de-energized mitochondria of the rat liver cells[12].

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