IDENTITY OF ANTIBIOTIC P-42-1 ELABORATED BY *ACTINOMYCES TUMEMACERANS* WITH KANCHANOMYCIN AND ALBOFUNGIN

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Antibiotic P-42-1 was isolated from the culture filtrate of *Actinomyces tumemacerans* strain INMI P-42, and its detailed physicochemical and biological properties were investigated. The antibiotic was obtained as yellow crystals and its molecular formula was estimated to be $C_{27}H_{24}N_2O_9$. It was found to be highly active on a wide variety of gram-positive bacteria and fungi, whereas much less active on gram-negative bacteria. The antibiotic was cytotoxic to HeLa cell cultures and exhibited antitumor activity on EHRLICH ascites tumor in mice. Antibiotic P-42-1 was found to be closely related to antibiotic BA-180265 A (kanchanomycin) and albofungin. According to structural studies on albofungin recently conducted by Russian workers, antibiotic P-42-1 proved to be identical with albofungin and kanchanomycin.

Actinomyces (*Streptomyces*) *tumemacerans* was isolated by Russian workers in 1962 from a soil sample collected in Askania Nova in the Ukrainian SSR[1]. The antibiotics produced by the strain were designated as P-42 A, P-42 B, P-42 E, and P-42 S[2]. According to TOKHTAMURATOV and SILAEV, P-42 A, B, E and S have been assumed to be cycloheximide, nystatin, rimocidin, and streptomycin or kanamycin, respectively[3].

In our preceding paper, detailed mycological studies on *A. tumemacerans* strain INMI P-42 were described. Cultural conditions for the production of these antibiotics were also investigated and one of the main antibiotics was isolated as yellow needles[4]. The antibiotic was found closely related or identical with BA-180265 A (kanchanomycin) reported by LIU *et al.* in 1963[5]. A. S. KHOKHLOV also informed us of the similarity of albofungin to antibiotic P-42-1 in 1971. Subsequently, the main antibiotic from strain INMI P-42 was highly purified, and its physicochemical and biological properties were studied.

Experimental

Physical and Chemical Properties of Antibiotic P-42-1

Antibiotic P-42-1 was obtained as yellow needle crystals which on gradual heating darken at 250~255°C but do not show a sharp melting point. The ultraviolet absorption maxima at 229 nm, 253 nm and 375 nm were ε 15,300, ε 15,600 and ε 9,200 respectively.

A shift to 388 nm was found in the alkaline alcohol but not in the acidic alcohol

(Fig. 1). The optical rotation is $[\alpha]_D^{25}-672°$ (c 0.5, pyridine). The behavior of the antibiotic in different chemical tests is given in Table 1. Antibiotic P-42-1 gives a positive reaction to ferric chloride solution, while it gives a negative response to MOLISCH, ELSON-MORGAN, anthrone and SAKAGUCHI reagents. Ninhydrin and biuret tests were also negative. The antibiotic does not reduce potassium permanganate.

The results of elementary analysis of antibiotic P-42-1 are as follow; Anal.

Fig. 1. Ultraviolet spectrum of antibiotic P-42-1.

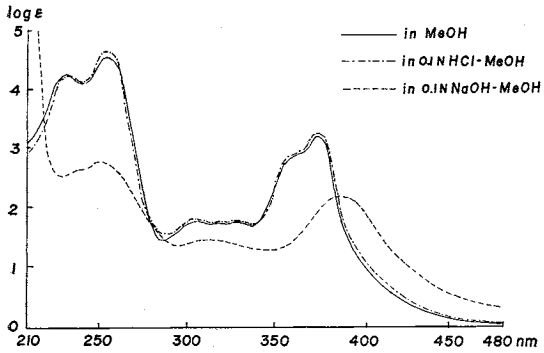


Table 1. Behavior of antibiotic P-42-1 towards different chemical tests

Chemical tests	Results
1. MOLISCH	Negative
2. Anthrone	Negative
3. ELSON-MORGAN	Negative
4. Ninhydrin	Negative
5. Biuret	Negative
6. SAKAGUCHI	Negative
7. Ferric chloride	Positive
8. $KMnO_4$	not reduced

Fig. 2. Mass spectrum of antibiotic P-42-1.

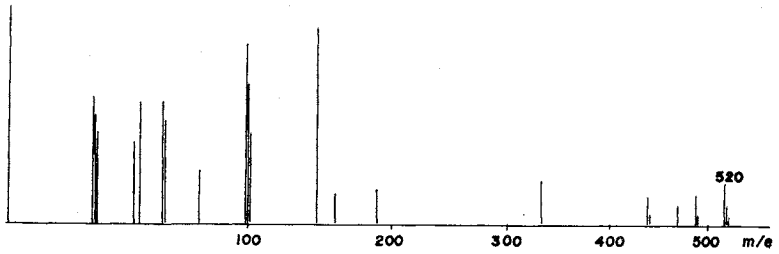
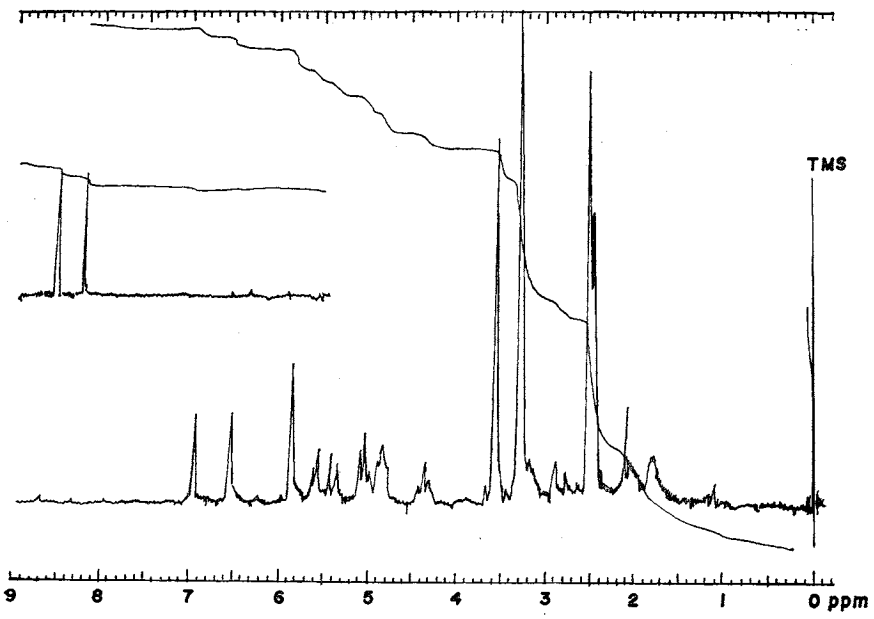


Fig. 3. NMR spectrum of antibiotic P-42-1 in D_6-DMSO.



Found: C 62.13, H 4.69, N 5.21, O 27.97. Calcd. for $C_{27}H_{24}N_2O_9$: C 62.31, H 4.65, N 5.38, O 27.66.

In the mass spectrum of antibiotic P-42-1, the peak at *m/e* 520, which is the highest numbered peak of all peaks, corresponds to $C_{27}H_{24}N_2O_9^+$ (Fig. 2). From the results of these experiments, the molecular formula of antibiotic is estimated to be $C_{27}H_{24}N_2O_9$.

The nmr spectrum of antibiotic P-42-1 measured by a Japan Electron Optics Lab. spectrometer (100 MHz) in dimethylsulfoxide using tetramethylsilane as an internal reference is shown in Fig. 3. Results mentioned above are consistent with those of A. I. GUREVICH *et al*[6].

Biological Properties of Antibiotic P-42-1

Antimicrobial activities; Antimicrobial spectra of the antibiotic were determined by the agar streak method. Antibiotic P-42-1 exerts notable activities against gram-positive bacteria.

The minimum inhibitory concentrations against these test organisms ranged from 0.05 to 2.5 mcg/ml. The antibiotic inhibited the growth of *Mycobacterium* species at a concentration of 1.0~10.0 mcg/ml. Much less activity was noted against gram-negative bacteria except for *Neisseria gonorrhoeae* which was inhibited at 0.25 mcg/ml. These results are shown in Table 2. Potent inhibitory effect of the antibiotic P-42-1 against fungi was also observed. The strains of *Candida albicans* showed high sensitivity to the antibiotic. Considerable

Table 2. Antibacterial spectrum of antibiotic P-42-1

Test organisms	Medium*	MIC (mcg/ml)
Staphylococcus aureus FDA 209 P	N	0.005
Staphylococcus aureus Rosa	N	0.25
Staphylococcus aureus Smith	N	1.0
Staphylococcus aureus 209 P (EM-R)	N	1.0
Staphylococcus albus	N	0.75
Staphylococcus citreus	N	5.0
Sarcina lutea	N	2.5
Bacillus subtilis PCI 219	N	0.75
Bacillus cereus	N	0.75
Streptococcus faecalis	B	7.5
Streptococcus pyogenes	B	0.25
Diplococcus pneumoniae	B	0.1
Neisseria gonorrhoeae	C	0.25
Corynebacterium dyphtheriae	N	0.25
Lactobacillus arabinosus	N	0.1
Mycobacterium sp. 607	N	1.0
Mycobacterium avium	N	10.0
Mycobacterium phlei	N	5.0
Serratia marcescens	N	50.0
Escherichia coli F_1	N	50.0
Escherichia coli F_1 (SM-R)	N	50.0
Pseudomonas aeruginosa	N	50.0
Proteus vulgaris	N	50.0
Shigella dysenteriae Shiga	N	50.0
Klebsiella pneumoniae	N	0.25
Nocardia brasiliensis	N	2.5
Nocardia somaliensis	N	0.75

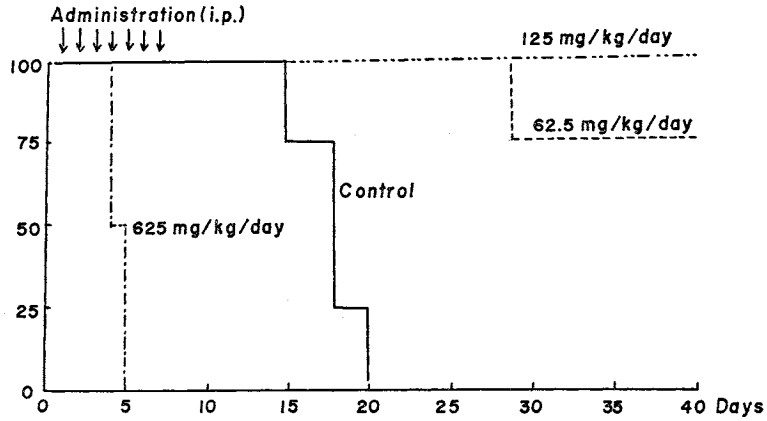
* N: nutrient agar, B: blood agar, C: chocolate agar

Table 3. Antifungal spectrum of antibiotic P-42-1

Test organisms	Medium*	MIC (mcg/ml)
Candida albicans IFM strain	S	0.075
Candida albicans ATCC 3170	S	0.0075
Candida albicans Nakagawa	S	0.0075
Candida albicans Saito	S	0.0075
Candida albicans 7 N	S	0.075
Candida albicans YU 1200	S	0.0075
Candida guiliermondii	S	0.05
Candida krusei	S	0.5
Candida parakrusei	S	0.5
Candida tropicalis	S	0.75
Candida stellatoidea	S	0.01
Cryptococcus neoformans	S	0.75
Torula rubra Saito	S	0.025
Penicillium glaucum	S	1.0
Aspergillus niger	S	1.0
Aspergillus oryzae	S	1.0
Mucor mucedo	S	0.25
Rhizopus nigricans	S	0.5
Saccharomyces cerevisiae Sake	S	0.075
Zygosaccharomyces salsus	S	0.0075

* S: SABOURAUD's agar

Fig. 4. Effects of antibiotic P-42-1 on EHRLICH ascites tumor.



activity was recorded against *Saccharomyces cerevisiae*, *Zygosaccharomyces salsus* and other fungi, as well. These results are shown in Table 3.

Toxicity: The LD_{50} for the antibiotic was found to be 399 mcg/kg when given to mice intravenously, and 2.0 mg/kg by intraperitoneal administration. Antibiotic P-42-1 was cytotoxic to HeLa cell culture at concentrations between 0.005 and 0.01 mcg/ml.

Effect on transplanted tumor: The effect of antibiotic P-42-1 against EHRLICH ascites tumor are illustrated in Fig. 4. When the treatment was started 24 hours after transplantation of 2.5×10^6 tumor cells, the increase of ascites and prolongation of life span were observed by daily intraperitoneal administration of more than 62.5 mcg/kg/day. All mice treated with 125 mcg/kg/day of the antibiotic survived for more than 40 days.

Discussion

The isolation of antibiotic BA-180265 A was first reported by LIU *et al.* in 1963. The antibiotic was named kanchanomycin, assigned NSC (Cancer Chemotherapy National Service Center U.S.A.) number 62773, and evaluated by the Western Cooperative Cancer Chemotherapy group, U.S.A.[7]

The mode of action was also investigated by FRIEDMAN *et al.*[8,9] However, the comprehensive physicochemical properties as well as biological activities have not appeared in the literature except for the description in the patent[10].

Antibiotic BA-180265 is reported to be comprised of two components. The relatively non-polar antibiotic was designated as BA-180265 A and polar one as BA-180265 B. The antibiotic P-42-1 is obviously closely related to antibiotic BA-180265 A. However, according to the results described above, antibiotic P-42-1 was found to be slightly different from the literature descriptions of antibiotic BA-180265 A in both physicochemical and biological properties. Elementary analysis of antibiotic P-42-1 and molecular weight determined by mass spectrum were different from those of BA-180265 A, the empirical formula of the latter compound being described as $C_{30}H_{22}N_2O_{10}$. As we could not obtain a reference sample of the BA-180265 A from the laboratory of Chas. Pfizer as well as from National Cancer Institute, direct side by side comparison of these two antibiotics was not carried out. However, it is our assumption that antibiotic P-42-1 is identical with BA-180265 A. After the crystallization of antibiotic P-42-1, KHOKHLOV and his co-workers suggested that antibiotic P-42-1 is closely related or identical with albofungin isolated by SOLOVIEVA and

RUDAYA from *Streptomyces albus* var. *fungatus* in 1959[11]. Recently, A. I. GUREVICH *et al.* reported determination on the structure of albofungin[6].

The results of the present investigation agree with the above assumption. It can be concluded that antibiotic P-42-1, albofungin, and antibiotic BA-180265 A (kanchanomycin) are identical to each other. Additional data on the biological activity of antibiotic P-42-1 confirmed its broad antimicrobial activity. LIU *et al.* first reported that broths from the source of antibiotic BA-180265 A showed only marginal activity against the standard mouse or rat tumor.

Subsequent experiments with BA-180265 A also failed to prove consistent or significant antitumor activity in a variety of animal tumor systems. The present investigation showed that EHRLICH ascites tumor system is fairly sensitive to this antibiotic. *Actinomyces tumemacerans* strain INMI P-42 was also found to produce albonoursin and a tetraene antifungal antibiotic in its culture filtrate. The details of their simultaneous production will be reported elsewhere.

References

1) KRASSILNIKOV, N. A. & A. D. KOVESHNIKOV : *Actinomyces tumemacerans* n. sp. A new species inducing disintegration of tumor in plant. Mikrobiologii 31 : 589~594, 1962
2) TOKHTAMURATOV, E.; A. B. SILAEV & S. M. KHODZHIBAEVA : Isolation of an antitumor substance from the culture fluid of *Actinomyces tumemacerans* P-42. Antibiotiki 9 : 205~208, 1964
3) TOKHTAMURATOV, E. & A. B. SILAEV : Recovery and purification of antibiotics produced by *Act. tumemacerans*. Antibiotiki 10 : 30~33, 1965
4) KUIMOVA, T. F.; K. FUKUSHIMA, S. KURODA & T. ARAI : Studies on *Actinomyces tumemacerans* strain INMI P-42 with particular reference to antibiotic production. J. Antibiotics 24 : 69~76, 1971
5) LIU, WEN-CHIN ; W. P. CULLEN & V. K. RAO : BA-180265 : A new cytotoxic antibiotic. Antimicr. Agents & Chemoth. -1962 : 767~771, 1963
6) GUREVICH, A. I.; M. G. KARAPETYAN, M. N. KOLOSOV, V. N. OMELCHENKO, V. V. ONOPRIENKO, G.I. PETRENKO & S. A. POPRAVKO : The structure of albofungin. Tetrahedron Letters 1972-18 : 1751~1754, 1972
7) BATEMAN, J. R.; A. A. MARSH & J. L. STEINFELD : Kanchanomycin (NSC-62773) : A phase I study. Cancer Chemother. Rep. 44 : 25~26, 1965
8) FRIEDMAN, P. A.; P. B. JOEL & I. H. GOLDBERG : Interaction of kanchanomycin with nucleic acid. Biochemistry 8 : 1535~1544, 1969
9) FRIEDMAN, P. A.; TING-KAI LI & I. H. GOLDBERG : Interaction of kanchanomycin with nucleic acid. Biochemistry 8 : 1545~1553, 1969
10) RAO, V.K.; P. BROOK, W. S. MARSH, WANAQUE & WEN-CHIN LIU : Antibiotic complex BA-180265 (A,B) and process for making same. U.S. Patent 3, 285, 814. Nov. 15, 1966
11) SOLOVIEVA, N. K. & S. M. RUDAYA : A description of a new antifungal antibiotic albofungin. Antibiotiki 4(6) : 5~10, 1959