A NEW ANTIBIOTIC, TALARON

Sir:

A new antifungal antibiotic, named talaron, has been isolated from the culture of a fungous strain M-3224, which was identified as *Talaromyces vermiculatus* (DANGEARD) BENJAMIN[1,2]. The antibiotic is water-soluble acidic polysaccharide containing nitrogen and phosphorus, and its molecular weight was estimated to be 7,000~8,000.

Talaron has strong fungicidal activity against filamentous dermatophytes. The antibiotic is produced in shaking or submerged cultures. For the production, the organism was precultured with shaking in a medium containing 1.0% glucose, 1.0% peptone, 1.0% corn steep liquor, 0.2% KH_2PO_4 and 0.1% $MgSO_4 \cdot 7H_2O$ at 26°C for 44 hours (pH 6.0). The culture was then transferred to a medium containing 8.0% glucose, 1.0% corn steep liquor, 0.65% $NaNO_3$, 0.5% $CaCl_2$, and 0.01% $MnSO_4$ (pH 3.5), and fermented submerging for about 50 hours at 26°C. Talaron produced in the broth was determined by paper disc-plate method using *Trichophyton asteroides* as a test organism on SABOURAUD's glucose medium (pH 6.0). The harvested broth (250 liters, 236 mcg/ml) was filtered, and 270 liters of acetone was added slowly to the filtrate (220 liters) with stirring at 5~8°C. After the mixture had been kept overnight at 5°C, the active precipitate was collected by centrifugation. Since the precipitate involved much of sparingly soluble material identified as calcium gluconate, talaron was extracted twice with each 15 liters of water. The water extract was reprecipitated with acetone. Similarly the second precipitate (1.5 kg) was treated with water for removal of calcium gluconate, and the extract was dialysed against water in a cellophane tube.

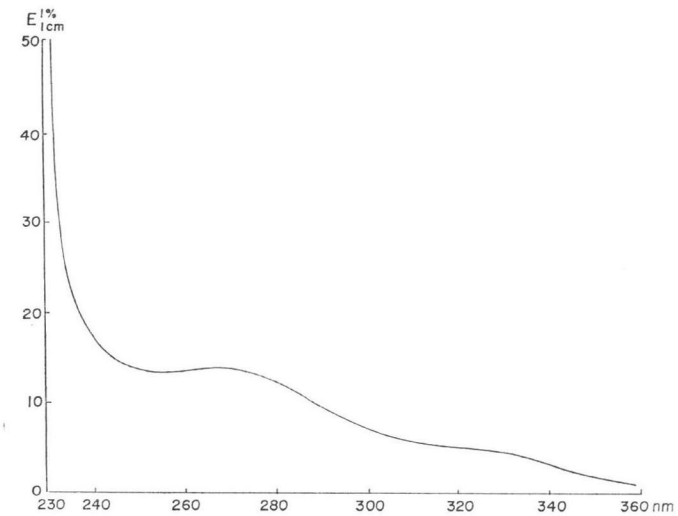
The inner solution was applied to DEAE-Sephadex (Pharmacia Fine Chemicals) column which had been equilibrated with 0.01 M phosphate buffer (pH 6.0), and the column was washed with the same buffer, then eluted with 0.2 M phosphate buffer (pH 6.0). The active eluates were collected and precipitated with acetone. The precipitate was dissolved in a small volume of water, and gel-filtered through the column of Bio-Gel P-30 (Bio-Rad Laboratories) with water.

The active eluates were combined and lyophilized yielding 5.2 g of powder (purity 96%, yield 9.6%). Further purification was made by repeating DEAE-Sephadex, and Bio-Gel treatments.

The purified talaron was pale yellow amorphous powder and the homogeneity was proved by ultracentrifuge using synthetic boundary method[3].

In an alternative procedure for the isolation, the adsorption of talaron on diatomaceous

Fig. 1. Ultraviolet absorption spectrum of talaron (in water)



earth was available, however, as the adsorbent had only quite limited capacity, it was not practically useful for large scale production.

Talaron thus obtained is pale yellow powder having a weakly acidic property, and darkens at 123~130°C. It is soluble in water, but insoluble in most organic solvents.

Anal. Found: C 44.10, H 5.76, N 9.36, O 38.81, P 1.97

$[\alpha]_D^{22}$ −324 (*c* 0.5, H_2O)

The molecular weight of talaron was estimated to be 7,000~8,000 by an ultracentrifuge. It exhibits positive phenol-H_2SO_4, anisaldehyde and carbazol-H_2SO_4 reactions, but negative ninhydrin, aniline-phthalate, ELSON-MORGAN and RYDON-SMITH reactions. The ultraviolet absorption spectrum in water (Fig. 1) shows a maximum at 273 nm ($E_{1cm}^{1\%}$ 15) with an inflection at 330 nm.

The infrared absorption spectrum (KBr

Table 1. Antimicrobial spectrum of talaron

Organisms	Method and medium	Minimum inhibitory concentration (MIC) (mcg/ml)	Organisms	Method and medium	Minimum inhibitory concentration (MIC) (mcg/ml)
Bacillus subtilis PCI 219	A N	>100	*Trichophyton asteroides*	A P	6.3
Staphylococcus aureus FDA 209 P	〃	>100	*Trichophyton rubrum*	〃	3.2
Sarcina lutea ATCC 1001	〃	>100	*Trichophyton schoenleini*	〃	>100
Mycobacterium ATCC 607	〃	>100	*Microsporum gypseum*	〃	3.2
Escherichia coli NIHJ	〃	>100	*Hormodendrum pedrosoi*	〃	6.2
Klebsiella pneumoniae PCI 602	〃	>100	*Piricularia oryzae* NI 4192	〃	25
Pseudomonas aeruginosa A_3	〃	>100	*Penicillium chrysogenum* Q 176	〃	3.2
Candida albicans	A P	12.5	*Aspergillus niger* ATCC 6275	〃	>100
Candida albicans	〃	50	*Aspergillus fumigatus* IAM 2612	〃	>100
Saccharomyces cerevisiae	〃	50	*Aspergillus* PQMD 82	〃	25
Mycotorula japonicus NI 6226	〃	50	*Trichophyton asteroides*	B S	0.16
Torula utilis	〃	25	*Trichophyton asteroides**	〃	0.08
Cryptococcus neoformans	〃	3.2	*Trichophyton interdigitale**	〃	0.16
Cryptococcus neoformans capsel	〃	6.3	*Trichophyton rubrum**	〃	0.08
Blastomyces dermatitidis	〃	100	*Microsporum gypseum**	〃	0.08
Trichoderma I-I ATCC 9645	〃	>100	*Hormodendrum pedrosoi**	〃	>10
			Sporotricum sp.*	〃	1.25
			Cryptococcus neoformans	〃	0.625

* Clinical isolate
Method: A; Agar dilution, B; Broth dilution
Medium: N; Nutrient, 37°C. 18 hours
P; Potato glucose, 30°C. 40 hours
S; SABOURAUD's glucose, 30°C, 40 hours

Fig. 2. Infrared absorption spectrum of talaron (KBr)

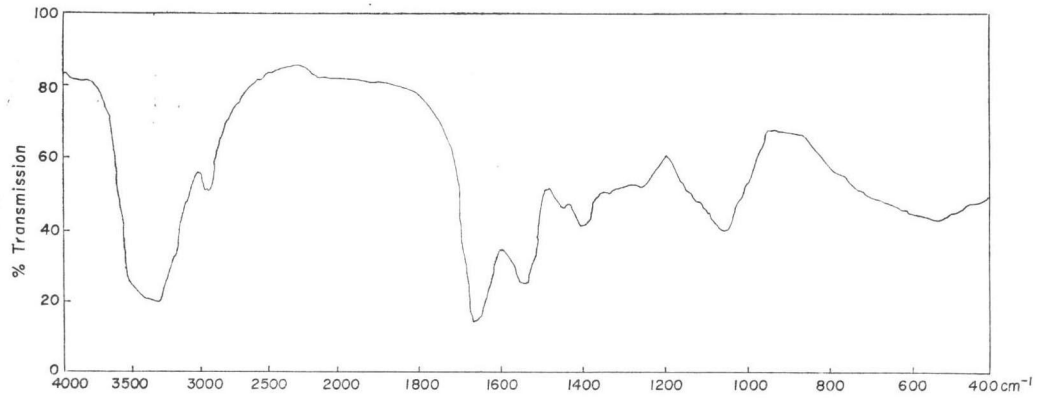


Fig. 3. Gas chromatogram of TMS derivatives prepared from talaron hydrolyzate

Instrument: Hitachi Gaschromatograph Model 063

Column: 3% SE-52 Chromosorb W(AW DMCG, 60~80 mesh); 0.3×200 cm stainless steel tube

Temperature: 100~300°C (10°C/min.)

Detection: FID. Hydrogen flame ionization detector

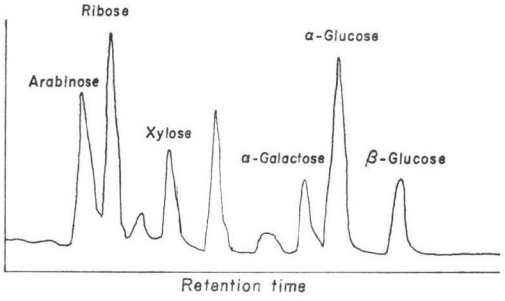


Table 2. Fungicidal effect of talaron

Time (hour)	Concentration (mcg/ml)					
	100	50	25	12.5	6.25	0
1	−	−	−	−	+	+
2	−	−	−	−	+	+
4	−	−	−	−	−	+
18	−	−	−	−	−	+
24	−	−	−	−	−	+

+: Presence of viable organisms
−: Absence of viable organisms

To the saline solutions involving various concentration of talaron, a spore suspension of *T. asteroides* was added (final spores: approximately 10^5/ml) and shaked at 30°C. After contact at intervals varying for 1 hour to 24 hours, two loopful of the fungal suspension was transplanted into SABOURAUD's broth and cultured for 10 days at 30°C. The presence of viable microorganisms in each sample was examined.

Table 3. Fungistatic and fungicidal MIC of talaron against spore and hyphae of *Trichophyton asteroides*

	Spore		Hyphae	
	Fungistatic M.I.C. (mcg/ml)	Fungicidal M.I.C. (mcg/ml)	Fungistatic M.I.C. (mcg/ml)	Fungicidal M.I.C. (mcg/ml)
Talaron	0.20	0.20	0.10	0.10
Griseofulvin	50	100	100	100

The sample solution was provided by dissolving an antibiotic in SABOURAUD's glucose medium and diluted with the same medium to prepare two serial 2-fold dilutions ranging from 0.005 to 1,000 mcg/ml. One serial 2-fold dilution was inoculated with the spores of *T. asteroides* (final concentration of spores: 3×10^8/ml) and another was inoculated with hyphae prepared by shaking in SABOURAUD's medium at 30°C for 40 hours (finally making 2% (v/v)-inoculum).

Both series were incubated for 7 days at 30°C, and minimal fungistatic concentration was determined through the observation of visible growth. Minimal fungicidal concentration was determined as follows: after the observation of fungistatic activity, a loopful culture was transplanted to potato glucose agar slant and cultured for 7 days at 30°C.

pellet) is given in Fig. 2.

On paper electrophoresis with cellulose acetate at 500 V for 60 minutes (0.1 M phosphate buffer, pH 7.0), talaron migrated 2.4 cm toward an anode. The acid hydrolysis of the antibiotic (1 N H_2SO_4, 105°C, 3 hours, in a sealed tube) resulted in the liberation of sugars, which were trimethylsilylated and then identified by means of gas chromatography.[4] As Fig. 3 shows, arabinose, ribose, xylose, galactose, glucose and other unidentified sugars were recognized.

Talaron is stable at neutral pH, but labile at acidic or alkaline pH at 40°C, and is inactivated over 60°C, an hour at pH 6.0.

The antibiotic is primarily active against filamentous dermatophytes, and moderately against yeast or yeast-like organisms, but has no antibacterial activity. The antimicrobial spectrum of talaron is shown in Table 1.

The fungicidal effect on *Trichophyton asteroides* was studied and the result suggested that talaron was fungicidal antibiotic like a mercurial preparation (Table 2). As mentioned above, talaron exhibited inhibitory activity against the spore germination of *T. asteroides*. The action on the hyphae was also examined in comparison with griseofulvin, and the result was expressed in terms of MIC value (Table 3). As the table shows, talaron is fungicidal against both the spore and the hyphae.

The antifungal activity was influenced by serum. The MIC value against *T. asteroides* was 0.1~0.2 mcg/ml as described above, but it was diminished to 6.25~12.5 mcg/ml in the medium containing 50 % serum of guinea pig, however, the effect of the serum was disappeared on heating at 70°C for 3 minutes.

Talaron showed cytotoxic effect at 1 mcg/ml on HeLa cells, and at 0.2 mcg/ml on mouse embryo fibroblast cells.

No antitumor activity was observed against EHRLICH ascites tumor in mice or rat-hepatoma AH 13.

Talaron is antigenic when injected in rabbit with incomplete FREUND's ajuvant, and the antibody was proved by means of precipitation reaction wiih the antibiotic.

Some of the polysaccharides with biological activities were reported to have interferon inducing activity[5], while talaron did not induce it in mice.

Intraperitoneal and intravenous LD_{50} values were calculated as 2.0 mg/kg and 15.0 mg/kg respectively.

From these chemical and biological properties, talaron is considered to be an unique antibiotic belonging to the polysaccharide, and it can be easily differentiated from known antibiotics.

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