Fradicin, an Antifungal Agent Produced by *Streptomyces fradiae*.* (17687)

E. Augustus Swart, Antonio H. Romano, and Selman A. Waksman.
From the Department of Microbiology, N.J. Agricultural Experiment Station, Rutgers University, New Brunswick, N.J.

Streptomyces fradiae, the organism that produces neomycin, an antibiotic which is active upon bacteria and actinomycetes, and which has but little activity upon true fungi, also forms an antibiotic which has no activity upon bacteria and actinomycetes and is active upon fungi. This antifungal agent is a fraction of the previously noted "Factor X"(1). As much as 30 to 50% of the antimicrobial activity of the broth was found to be due to this factor. When less air was supplied during growth of *S. fradiae* in submerged culture, the concentration of this factor was increased to 66.6% in bacto-peptone broth and to 69.2 in soya peptone medium. Further studies brought out the fact that factor X is made up of two entities, one of which is an antibacterial and the other an antifungal substance. The latter has been designated as *fradicin.* It is active against a variety of fungi, including various saprophytic and pathogenic forms, such as *Penicillium notatum, Aspergillus niger, Ceratostomella ulmi, Trichophyton mentagrophytes* and *Candida albicans,* as shown in Table I. The amount of material necessary to inhibit the growth of

TABLE I.
Antibiotic Spectrum of Fradicin.

Organism	Units/g
Staphylococcus aureus	0
Bacillus mycoides	0
" *subtilis*	0
Escherichia coli	0
Streptomyces griseus	0
Trichophyton mentagrophytes	300000
Trichoderma 55	250000
Aspergillus niger 13	400000
Fusarium sp.	50000
Penicillium notatum 50	750000
Ceratostomella ulmi	>1000000
Candida albicans	250000

* Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University,-The State University of New Jersey, Department of Microbiology.

1. Swart, E. A., Hutchison, D., and Waksman, S. A., *Arch. Biochem.,* 1949, v24, 92.

P. notatum in 1 ml of nutrient agar, as determined by the agar-streak method(2) is taken as a unit. The ratio of activity against different fungi may change with the composition of the medium, possibly because of the limitations of the method. Its usual activity in culture broth is 2 to 4 times as great against *P. notatum* and *C. ulmi* as against *T. mentagrophytes* and *C. albicans.* The presence of glucose in the test medium reduces the activity of the antibiotic, either by reducing its potency or by stimulating growth of the test organisms.

Production and isolation of fradicin. Media containing glucose give good production of fradicin, as shown in Table II. Addition of $CaCO_3$ is not necessary, since the medium does not become too acid even with 1% glucose. Fradicin is removed from the broth on acidification to pH 2.0-2.5 and treatment with active charcoal. Since fradicin is readily soluble in butanol at a pH range of 2.0 to 8.0, this solvent is used for further extraction. Fradicin can also be removed directly from the broth by acidification to pH 2, filtering with the aid of supercel, and eluting the supercel with sodium hydroxide solution at pH 8.0. The final isolation of fradicin from the medium and further concentration can best be carried out as follows:

Two liters of soya-peptone culture filtrate with an activity of 75 *P. notatum* units per ml is stirred for 30 minutes with one liter of *n*-butanol at pH 7.0. The alcohol phase is concentrated *in vacuo* to dryness and taken up in 95% ethanol. The alcoholic solution is concentrated to small volume and added dropwise to acetone. The solution is concentrated *in vacuo* and added dropwise to petroleum ether. The supernatant liquid phase is discarded. The oil is dissolved in *t*-butanol and lyophilized. A light tan-colored powder, hygro-

2. Waksman, S. A., and Reilly, H. C., *Ind. Eng. Chem., Anal. Ed.,* 1945, v17, 556.

TABLE II.
Influence of Composition of Medium upon Formation of Fradicin.
Shake cultures, 5 days incubation.

Medium	Dilution units per ml		
	P. notatum	*C. albicans*	*C. ulmi*
Soya peptone	100	50	200
Nutrient broth	<10	<10	50
Yeast-glucose	100	50	>200
" " + $CaCO_3$	75	35	175
NZ amine	50	10	100
" + 1% glucose	100	65	200
" + " + $CaCO_3$	75	30	100

TABLE III.
Stability of Fradicin.

pH	Time, hr	Temp.,°C	Dilution units per ml		
			P. notatum	*C. ulmi*	*T. mentagrophytes*
Original potency of broth			50	75	20
1.5	72	25	30	30	5
2.0	72	25	30	30	5
3.0	72	25	40	50	10
7.0	72	25	50	75	20
2.0	1/6	60	<5	<5	<5
7.0	1/2	100	50	50	20

scopic in nature and having a potency of 312,000 *P. notatum* dilution units, is obtained. The yield is about 50 mg per liter of culture, with a recovery of 20 to 30% of the original activity.

Solubility and stability of fradicin. Fradicin is soluble in various alcohols (methanol, ethanol, butanols and amyl alcohols) and in chloroform. It is insoluble or very sparingly soluble in ether, carbon tetrachloride, benzene, acetone, and petroleum ether. Although only slightly soluble in water, concentrated alcoholic solutions can be dropped into a large volume of water without precipitation. Fradicin is stable at 100°C for 30 minutes in a solution of pH 7.0. At pH 2.0, 10 minutes at 60°C is sufficient to destroy the antibiotic completely. At room temperature, fradicin is stable at pH 7.0, but gradually loses its activity at acid reactions. This is brought out in Table III.

Comparison of fradicin with other antifungal antibiotics. Among the other antibiotics that possess strong antifungal properties, one is tempted to compare fradicin with streptothricin, on the one hand, and with actidione and antimycin A. on the other. Since streptothricin also possesses marked antibacterial properties and is so different in its solubility properties, further comparison is precluded. The solubility properties and the lack of stability of actidione above pH 7 differentiate this antibiotic from fradicin and antimycin A. Finally, the ultraviolet absorption spectrum of a sample of fradicin having a potency of 800 units per mg showed no maxima at either 227 or 330 mμ (Fig. 1); this definitely characterizes fradicin in contradistinction to antimycin A(3,4). The antimicrobial spectra of these 3 antibiotics are also markedly different.

Summary. An antibiotic, designated as fradicin and active only upon fungi, was isolated from the culture filtrate of the neomycin-producing *Streptomyces fradiae.* Fradicin is characterized by an antifungal spectrum, which includes saprophytes as well as plant and animal pathogens. It is stable to 100°C for 30 minutes at pH 7.0. At more acid reactions, it is gradually destroyed. It is soluble

3. Dunsbee, B. R., Leben, C., Keitt, G. W., and Strong, F. M., *J. Am. Chem. Soc.*, 1949, v71, 2436.

4. Leben, C., and Keitt, G. W., *Phytopath.*, 1948, v38, 899.

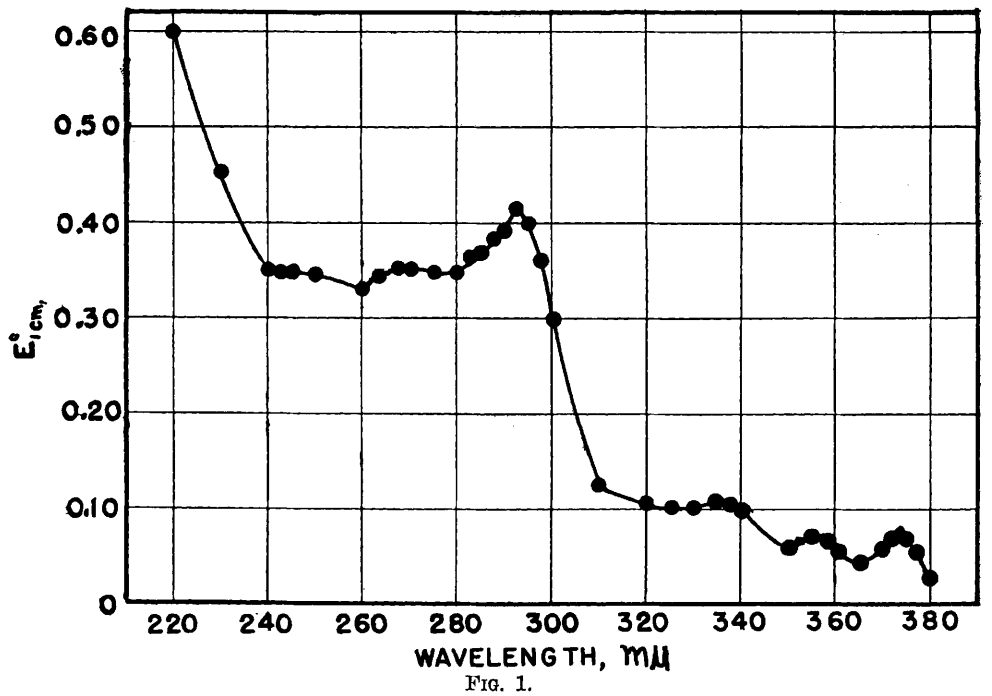

FIG. 1.
Absorption Spectrum of Fradicin.
c = 0.0174 g/l in absolute methanol.
E^c_{1cm} = extinction at conc. c.

in a number of organic solvents and has a characteristic absorption spectrum which differentiates it from other antibiotics.

The authors wish to express their sincere appreciation to Dr. Dorris Hutchinson and Mr. H. Lechevalier, of this laboratory, for assisting in some of the experimental work.

Received February 8, 1950. P.S.E.B.M., 1950, v73.

Does the Excretion of Diethylstilbestrol Glucuronide Influence the Venning Determination for Urinary Pregnanediol? (17688)

O. WATKINS SMITH AND SARA SCHILLER. (Introduced by George Van S. Smith.)

From the Fearing Research Laboratory, Free Hospital for Women, Brookline, Mass., and the Department of Gynecology, Harvard Medical School, Boston, Mass.

An apparent increase in the excretion of pregnan-3(α), 20(α)-diol as a result of the prophylactic administration of diethylstilbestrol to a pregnant woman was first reported in 1946(1). This observation has been amply confirmed in this laboratory(2,3,4) and by other investigators(5), provided the pregnanediol is measured as the sodium glucuronide (NaPG) by the Venning procedure(6).

1. Smith, O. W., Smith, G. V. S., and Hurwitz, D., *Am. J. Obst. and Gynec.*, 1946, v51, 411.

2. Smith, O. W., and Smith, G. V. S., *West. J. Surg.*, 1947, v55, 313.

3. Smith, O. W., *Am. J. Obst. and Gynec.*, 1948, v56, 821.

4. Smith, O. W., to be published.

5. Davis, M. E., and Fugo, N. W., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 436.

6. Venning, E. H., *J. Biol. Chem.*, 1937, v119, 473.