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The Antibiotic Action of *Tillandsia usneoides* (Spanish Moss).

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It is now well established that certain bacterial organisms, fungi and algæ[1] produce antibiotic substances for various bacteria and fungi. Extracts of certain plants,[2,3] including lichens, and the heartwood of the Western Red Cedar[4] also have recently been shown to possess inhibitory properties. So far as we know, however, no epiphyte has previously been tested for inhibitory activity. The following is a report of tests that demonstrate that extracts of *Tillandsia usneoides* (Spanish Moss), an epiphyte, are also inhibitory to certain organisms.

The *Tillandsia* used in this work was taken from trees in the neighborhood of Charleston, S.C. We found that alcoholic, acetone, or chloroform extracts of this plant were frequently inhibitory to *Staphylococcus aureus*. On further study, we discovered that most of the antibiotic substance was present in certain brownish parts of the plant, and that preparations of relatively high potency could be obtained by subjecting these parts to extraction with acetone or chloroform.

Methods. The method which consistently gives the most potent extracts is as follows: The plant is cut up into fragments of 0.5 to 1.5 cm in length. In order to separate the brown from the green inactive parts, the fragments are stirred thoroughly in 95% alcohol. The heavier green fragments sink to the bottom of the container, while the brown lighter ones rise to the surface of the liquid and may be skimmed off and dried at 37°C. The weight of this dried material is approximately one-tenth of that of the original plant. The material is then shaken with acetone or chloroform for several hours, and filtered through one layer of gauze. The acetone or chloroform extract thus obtained is evaporated off at room temperature. The slight amount of brownish or greenish yellow residue on the evaporating dish is taken up in 2 cc of alcohol for every gram of brown material originally extracted. The cloudy fluid obtained is centrifuged, and the clear olive green or yellow supernatant thus obtained is tested for antibiotic activity. The brown Tillandsia fragments can be re-extracted several times with decreasing yields of inhibitory substances.

Most of the tests were carried out against *Staphylococcus aureus* H. (Oxford strain). A serial dilution method was employed similar to that used by McKee, Rake, and Menzel.[5] Bacto-nutrient broth was used throughout these experiments. The highest dilution that completely inhibited growth in two days at 37°C was taken as the endpoint. One inhibiting unit of the Tillandsia extract was arbitrarily taken as the minimum quantity of its alcoholic solution which when added to 1 cc of nutrient broth inhibited completely the growth of the Staphylococcus for 2 days. Suitable controls for the alcoholic content of the extracts were included.

Results. Our Tillandsia extracts, prepared as described above, contain from 400 to 1600 units per cc. Other organisms which had the same order of sensitivity to our extract as the Staphylococcus were Pneumococcus I, II, and III, *Streptococcus hemolyticus* C 203, and *Cryptococcus hominis*. *H. influenzæ* Type B and *Candida albicans* were less sensitive. In these latter experiments, appropriate media for the growth of the organisms and for dilution in the inhibition tests were utilized. Our extract in 1 to 50 dilution (containing the highest concentration of alcohol permissible)

[1] Pratt, R., *Science*, 1944, **99**, 351.

[2] Osborn, E. M., *Brit. J. Exp. Path.*, 1943, **24**, 227.

[3] Lucas, E. H., and Lewis, R. W., *Science*, 1944, **100**, 597.

[4] Southam, C. M., and Ehrlich, J., *Phytopathology*, 1943, **33**, 517.

[5] McKee, C. M., Rake, G., and Menzel, A. E. O., *J. Immunol.*, 1944, **48**, 259.

had no inhibitory effect on the growth of *H. influenzæ* Type A, *Ps. pyocyaneus* (2 strains), *E. coli* (2 strains), and *B. proteus*.

Summary. A method for extracting an antibiotic substance from *Tillandsia usneoides* (Spanish Moss) is described. The crude extract is effective against all the gram-positive bacteria tested and against *Cryptococcus hominis*.

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Antifungal Properties of Clavacin.*†

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The value of certain antibiotic substances, such as penicillin and tyrothricin, for the treatment of many bacterial infections is well established, although the vast majority of such agents are considered too toxic for therapeutic use. It is of especial interest to note, however, that an agent such as tyrothricin, which is toxic on parenteral injection, is nontoxic when given orally or applied externally. Thus, in the treatment of certain types of local infections, tyrothricin has met with remarkable success.[1]

The majority of fungous infections are both local and superficial in character. It follows, therefore, that toxicity upon injection does not necessarily disqualify a possible therapeutic agent. Since many fungi are known to be susceptible to the action of antibiotic substances, and since there is a paucity of satisfactory therapeutic agents for the control of mycoses, it appears that the effects of antibiotic agents on the pathogenic fungi should be investigated.

The following report deals with the effect of clavacin on 3 representative species of fungi, viz., *Monilia albicans, Oidium asteroides,* and *Trichophyton gypseum.*‡

In the early part of this work the clavacin was produced in this laboratory according to the methods of Waksman, Horning, and Spencer,[2] employing a strain of *Aspergillus clavatus* known to produce a high yield of clavacin. During the course of the investigation, crystalline clavacin became available and the tests were repeated using 4 samples of this material obtained from other sources.§

Fungistatic Effects. The ability of clavacin to inhibit the growth of fungi was determined by growing the test organisms in the presence of graded concentrations of this agent. Measured amounts of a liquid medium‖ were placed in flasks and sterilized in the autoclave, after which the portions of medium were restored to volume by the addition of sterile distilled water. To each flask was then added, aseptically, the desired quantity of an aqueous solution of clavacin. The 2% stock solution of this compound was self-sterilizing. After thorough agitation of the mixture of the medium and clavacin solution, 25 ml amounts were aseptically dispensed into sterile flasks.

* Clavacin is also known as clavatin, clavaformin and patulin.

† These investigations were supported in part by a grant supplied by Parke, Davis and Company, Detroit, Michigan.

1 Hoogerheide, J. C., *Bot. Rev.*, 1944, **10**, 599.

‡ The culture of *T. gypseum* (granular type) was obtained through the courtesy of Dr. C. W. Emmons, U. S. Public Health Service. The others were taken from the culture collection of this laboratory.

2 Waksman, S. A., Horning, E. S., and Spencer, E. L., *J. Bact.*, 1943, **45**, 233.

§ One sample of crystalline clavacin was kindly supplied by Parke, Davis and Company and 3 samples by the Mallinckrodt Chemical Works, St. Louis, Mo.

‖ Constituents: $NaNO_3$ 2.0 g, KH_2PO_4 1.0 g, KCl 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $MnSO_4 \cdot 4H_2O$ 0.01 g, glucose 20.0 g; peptone 5.0 g, and distilled water to make 1 liter.