

focused with 100-fold magnification on the microscope. Then a drop of 0.1 *N* HCl was introduced onto the microscope slide along the side of the cover glass. After a slight adjustment of the focus and the intensity of the light source, time-lapse photomicrographs were taken. The photomicrographs in Fig. 3 demonstrate a series of time-lapse photomicrographs taken of the same microscopic field without any stirring or agitation. After the solvent was in contact with the crystal for approximately 1 min., the hexagonal shape of the drug was still recognizable. After 10 min. of contact time, progressive transformation of the hexagonal to the rod-shaped crystals was observed and recorded with the photomicrographs. Under the experimental conditions employed the complete transformation into the rod-shaped crystals was observed to take place within 15 min.

In order to prepare enough quantity of samples for physical characterization, an excess of the drug beyond its equilibrium solubility was introduced into approximately 20 ml. of 0.1 *N* hydrochloric acid contained in a Petri dish at 22°. The drug and the medium were kept on the bench without agitation to mimic the condition of the microscopic slide. After the drug was in contact with the medium for 15 (Sample E) and 30 (Sample F) min., portions of residual solid materials were filtered rapidly through a filter, dried under vacuum at 40° for 24 hr. and characterized again by means of photo-

micrograph, melting point, infrared spectra, and X-ray diffraction pattern, as compiled in Tables I and II and Fig. 2. Inspection of physical characteristics of Samples E and F, as compared with that of Samples B, C, and D, indicated that the *in situ* crystalline transformation observed by dissolution method at 500 r.p.m. for a few minutes is similar to that attainable by photomicrographic or Petri dish methods without agitation at the end of 15 min. A detailed report on this investigation and the *in vitro* to *in vivo* relationship for this compound will be presented in the future (2).

The photomicrographic technique employed in this study for observing and recording the *in situ* crystalline transformation of the drug is simple, rapid, and straightforward. Meaningful results can be obtained within a short period of time.

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SONG-LING LIN

LEON LACHMAN

Pharmacy Research and Development Division  
Development and Control Department  
CIBA Pharmaceutical Company  
Summit, NJ 07901

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## Antibacterial Activity of *Solanum carolinense* L.

**Keyphrases** □ *Solanum carolinense*—antimicrobial extract □  
Antimicrobial spectrum—*S. carolinense*

Sir:

In a screening procedure for antibacterial activity of several crude botanical materials, small pieces of air-dried fruit of *Solanum carolinense* L. (horse-nettle) produced marked inhibition zones on blood-agar plate cultures of recent hospital isolates of *Pseudomonas* species. Lysis of red cells occurred around the samples placed on the plates. This study has been undertaken to define the antimicrobial spectrum and to isolate the active constituents of this plant. This preliminary report summarizes the antimicrobial spectrum of aqueous extracts of *S. carolinense*.

Frisby *et al.* (2) have reported inhibitory activity against *Mycobacterium* species in aqueous extracts of the leaves of *S. carolinense*. A galenical preparation of *S. carolinense* was recognized in NF V. It was formerly used in the treatment of epilepsy.

Extracts were prepared by macerating air-dried ground fruit (25 g.) for 24 hr. with 100 ml. 1% acetic acid. The resulting extract was filtered and ethanol (100 ml. of 95%) added to the filtrate and the resulting precipitate removed by filtration and discarded. The filtrate was shaken with three successive 50-ml. portions of chloroform. The hydroalcoholic layer was then brought to pH 9 with 10% NaOH and again shaken with three successive 50-ml. portions of chloroform. The hydroalcoholic fraction was brought to pH 7 with 10% acetic acid and evaporated to dryness on antibiotic sensitivity disks in an oven at 60°. The resulting disks were placed on agar plates streaked with the test organisms. Sabouraud dextrose agar,<sup>1</sup> Sauton's agar (1), and trypticase-soy agar<sup>1</sup> were used for the cultivation of the fungi, the *Mycobacterium* species, and the remaining bacterial species, respectively. Disks were also placed on blood-agar plates to detect any remaining hemolytic activity. Widths of inhibition zones were measured from the edge of the disks to the edge of the region of visible microbial growth. Zones were measured when sufficient confluent growth appeared on the surface of the agar.

The sensitivities of the responding test organisms

<sup>1</sup> Difco Laboratories, Detroit, MI 48201

**Table I**—Response of Test Organisms Sensitive to Aqueous Extracts of *S. Carolinense* L.

Test Organism	Width of Zone, (mm.)
<i>Mycobacterium smegmatis</i>	2.3
<i>M. marinum</i>	1.8
<i>M. phlei</i>	2.3
<i>Penicillium</i> sp., isolate No. 1	1.3
<i>Pseudomonas aeruginosa</i> , isolate No. 1	1.3
<i>P. aeruginosa</i> , isolate No. 2	2.3
<i>Pseudomonas</i> sp.	2.3
<i>Proteus rettgeri</i>	1.3
<i>P. mirabilis</i>	1.3
<i>P. morgani</i>	2.2
<i>Aerobacter cloacae</i>	2.0
<i>Aerobacter</i> sp.	2.1
<i>Klebsiella pneumoniae</i>	1.2
<i>Escherichia coli</i> , isolate No. 1	1.8
<i>E. coli</i> , isolate No. 2	2.3
<i>E. freundii</i>	3.8
<i>S. aureus</i> , isolate No. 2	1.8 <sup>a</sup>

<sup>a</sup> In this test, pinpoint-sized colonies occurred within the zone of general inhibition.

appear in Table I. Of the fungi tested, only one isolate of *Penicillium* species was found to be sensitive. The fungal organisms tested were *Saccharomyces cerevisiae*, *Aspergillus fumigatus*, *Candida albicans* (three isolates), *Candida* species, *Mucor* species, and *Penicillium* species (three isolates). Of the mycobacteria tested, only *M. butyricum* was resistant. Remaining bacterial species found to be resistant were *Pseudomonas aeruginosa* (one isolate), *Bacillus cereus*, and *Staphylococcus aureus* (one isolate).

With the exceptions of the activity observed against one isolate of *Penicillium* species and one isolate of *Staphylococcus aureus*, aqueous extracts of fruits of *S. carolinense* possess selective inhibitory activity against certain Gram-negative bacteria. These extracts also possess antimycobacterial activity similar to that reported by Frisby *et al.* (2) for aqueous extracts of leaf of *S. carolinense*. Since the steroid alkalines present in *S. carolinense* should have partitioned into chloroform at alkaline pH, and phenolic compounds should have partitioned into chloroform at acidic pH, antibacterial activity is probably not due to these compounds. Since the extracts retain activity after heating at 60° for at least 36 hr., the active compounds must be fairly stable. Hemolysis was not produced by the chloroform treated extracts on blood agar plates. Further fractionation of the active extracts is presently underway.

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OLIN M. PITTS\*  
HUGH S. THOMPSON  
J. H. HOCH

\*Department of Microbiology  
Department of Biology and Pharmacognosy  
Medical College of South Carolina  
Charleston, SC 29401

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