Scopafungin, a Crystalline Antibiotic Produced by *Streptomyces hygroscopicus* var. *enhygrus* var. *nova*

**LEROY E. JOHNSON AND ALMA DIETZ**

*Infectious Diseases Research, The Upjohn Company, Kalamazoo, Michigan 49001*

Received for publication 13 May 1971

Scopafungin (U-29,479) is a crystalline, nonpolyenic antimicrobial agent obtained from the culture broth of *Streptomyces hygroscopicus* var. *enhygrus* var. *nova* UC-2397. Scopafungin inhibits, in vitro, a variety of pathogenic fungi, yeasts, and gram-positive bacteria.

**Streptomyces hygroscopicus** var. *enhygrus* var. *nova* produces an antibiotic designated scopafungin (U-29,479). *S. hygroscopicus* var. *enhygrus* also produces the polyenic endomycin complex reported for *S. endus* NRRL-2339 (Gottlieb and Carter, U.S. Patent 2,746,902, 1956). Scopafungin was isolated as a white crystalline, nonpolyenic compound from the complex produced by the new culture. It is soluble in methanol, ethanol, n-propanol, and glacial acetic acid but is only slightly soluble in water, acetone, methylene chloride, chloroform, butanol, and ether.

The isolation and chemical properties of scopafungin will be described by M. E. Eberg (in preparation). Studies on the mode of action of this antibiotic will be reported by F. Reusser (in preparation).

This paper describes the organism, the fermentation, the paper chromatogram characterization, and some of the biological properties of the antibiotic.

**MATERIALS AND METHODS**

**Culture.** *S. hygroscopicus* var. *enhygrus* was characterized by the methods cited by Dietz (1) and by Shirling and Gottlieb (8). *S. hygroscopicus* var. *enhygrus* UC 2397 was compared with the type species *S. hygroscopicus* (Jensen) Waksman, CBS, UC 2317 and the type species *S. endus* NRRL 2339, UC 2020.

**Medium.** Seed flasks were inoculated with spore preparations of the culture which were maintained in soil. The culture was incubated at 28 C for 72 hr in a seed medium consisting of, per liter: 25 g of glucose monohydrate (Cerelose) and 25 g of Pharmamedia (Trader Oil Mill Co., Fort Worth, Tex.). The vegetative seed was used at a rate of 5% to inoculate a fermentation medium consisting of, per liter: 60 g of glucose monohydrate, 20 g of Hi-starch (Illinois Cereal Mills, Inc., Paris, Ill.), 4 g of CaCO<sub>3</sub>, 4 g of NaNO<sub>3</sub>, 20 g of Pharmamedia. The pH was adjusted to 7.2 before sterilization. Shaken-flask fermentations were run in 500-ml Erlenmeyer flasks containing 100 ml of medium and for optimal yields were incubated at 28 C on a Gump rotary shaker operating at 250 rev/min with a 2.5-inch (6.4 cm) stroke.

**Antibiotic assay procedure.** The antibiotic concentrations were determined with a disc-plate agar diffusion assay. The antibiotic standards and all samples were prepared in a 20% dimethylformamide (DMF) solution. Samples (0.08 ml) and dilutions (also made in 20% DMF) were placed on 12.7-mm paper discs and were assayed against *Saccharomyces cerevisiae* (UC-1337). A liquid suspension of *S. cerevisiae* was inoculated into an agar medium consisting of, per liter: 10 g of dextrose, 2.5 g of yeast extract (BBL), 1.0 g of KH<sub>2</sub>PO<sub>4</sub>, and 17.5 g of agar; final pH, 5.65. The zones of inhibition of growth were measured after incubation for 18 hr at 37 C. The antibacterial activity was expressed as micrograms of scopafungin standard per milliliter run by a standard curve assay. The range of assay is between 31.25 and 250 µg per ml.

The antibacterial in vitro spectrum was determined by twofold dilution end points in Brain Heart Infusion (Difco) broth. Readings were made after 20 hr of incubation at 37 C. The antifungal in vitro spectrum was determined by the agar dilution plate assay of Whiffen (12) with readings made after 72 hr at 28 C.

Scopafungin was differentiated from other antibiotics by its antibacterial and antifungal spectra, by its chemical properties, and by paper chromatography. The antibiotic was spotted on Whatman no. 1 filter paper and developed without prior equilibration by use of the descending method and the solvent systems listed in Fig. 1. Antibiotic activity was located by plating the developed strips on trays of agar seeded with *S. cerevisiae*.

**RESULTS AND DISCUSSION**

**Taxonomy.** *Streptomyces hygroscopicus* var. *enhygrus* Dietz var. *nova*.

**Color characteristics.** Aerial growth white to gray-white or gray-cream to gray. Moist, black, hygroscopic patches on some media. Melanin-negative. Appearance on Ektachrome is given in Table 1. Reference color characteristics are given in Tables 2 and 3. The cultures may be placed in
TABLE 1. Appearance of Streptomyces hygroscopicus cultures on Ektachrome

| Agar medium* | Determination | S. hygroscopicus var. enkrygt CBS 2397 | S. hygroscopicus CBS 2317 | S. endus NRRL 2339, UC 2020 |
|--------------|---------------|----------------------------------------|--------------------------|-----------------------------|
| Bennett's    | R             | Gray-white                            | Gray                      | Gray                        |
| Czapek's sucrose | R            | Yellow-tan                             | Yellow                    | Tan                         |
| Maltose-tryptone | R            | Yellow                                | Yellow-gray               | Yellow-grey                 |
| Peptide-iron  | S             | Yellow                                | Yellow                    | Trace grey-white            |
| 0.1% Tyrosine | S             | Yellow                                | Trace grey-white          | White                       |
| Casein starch | R             | Yellow                                | White                     | Pale Yellow                 |

TABLE 2. Reference color characteristics of Streptomyces hygroscopicus cultures

| Agar medium       | Determination | S. hygroscopicus var. enkrygt CBS 2397 | S. hygroscopicus CBS 2317 | S. endus NRRL 2339, UC 2020 |
|-------------------|---------------|----------------------------------------|--------------------------|-----------------------------|
| Bennett's         | R             | b(m)                                    | 1f(g)                    | 2g(m)                       |
|                   | P             | 2g(c)(m)                                | 2g(c)                    | 2e(g)                       |
|                   |               |                                        |                          | 263m, 264g                  |
|                   |               |                                        |                          | 263m, 264g                  |
|                   |               |                                        |                          | 90gm                        |
|                   |               |                                        |                          | 90gm                        |
|                   |               |                                        |                          | 112m, 122g                  |
|                   |               |                                        |                          | 94m, 90g                    |
|                   |               |                                        |                          | 91g, 94g, 106g              |
| Czapek's sucrose  | R             | 2f(g)                                   | 3f(g)                    | 4g, 112g                    |
|                   | P             | 1f(e)(m)                                | 2f(c)                    | 93g                         |
|                   |               |                                        |                          | 63g                         |
|                   |               |                                        |                          | 112m, 122g                  |
|                   |               |                                        |                          | 264g                        |
|                   |               |                                        |                          | 264g                        |
| Maltose-tryptone  | R             | b(m)                                    | 1c(m)                    | 1d(g)                       |
|                   | P             | 1c(m)                                   | 2c(g)                    | 2b(g)                       |
|                   |               |                                        |                          | 263m, 264g                  |
|                   |               |                                        |                          | 90gm                        |
|                   |               |                                        |                          | 92m, 93m                    |
|                   |               |                                        |                          | 92m, 93m                    |
| Yeast extract-malt extract (ISP-2) | R | 2f(g)                                   | 1d(c)                    | 3f(e)(m)                    |
|                   | P             | 1e(c)                                   | 2g(c)                    | 2e(g)                       |
|                   |               |                                        |                          | 94g, 112g                   |
|                   |               |                                        |                          | 90gm                        |
|                   |               |                                        |                          | 121m, 122g                  |
|                   |               |                                        |                          | 63g                         |
|                   |               |                                        |                          | 91g, 94g, 106g              |
| Oatmeal (ISP-3)   | R             | 2m(c)(m)                                | 2e(g)                    | 1d(c)                       |
|                   | P             | 3f(e)(g)                                | 2d(c)                    | 1i(e)(g)                    |
|                   |               |                                        |                          | 63g                         |
|                   |               |                                        |                          | 93g                         |
|                   |               |                                        |                          | 121m, 122g                  |
|                   |               |                                        |                          | 121m, 122g                  |
|                   |               |                                        |                          | 104g, 121m                  |
| Inorganic-salts starch (ISP-4) | R | 3f(e)(g)                                | 2f(e)(g)                 | 1b(c)                       |
|                   | P             | 2b(c)(m)                                | 2d(c)                    | 1c(a)(g)                    |
|                   |               |                                        |                          | 63g                         |
|                   |               |                                        |                          | 93g                         |
|                   |               |                                        |                          | 121m, 122g                  |
|                   |               |                                        |                          | 104g, 121m                  |
| Glycerol-asparagine (ISP-5) | R | 2f(e)(m)                                | 2b(c)(m)                 | 1c(a)(g)                    |

* S, surface; R, reverse; P, pigment; (m), matte; (g), glossy.

** See Table 3 for color names.

the white (W) and gray (GY) color series of Tresner and Backus (10).

**Microscopic characteristics.** Sporophores in tight spirals. Sporophores spiral in the sense of Pridham et al. (7). Spores frequently forming dark, hygroscopic patches. Spores smooth with an irregular possibly warty surface by direct electron microscope examination. Spore surface rugose when examined by the carbon replication method of Dietz and Mathews (2, 3). Coremia present on some media.

**Cultural and biochemical characteristics.** Cultural and biochemical characteristics are described in Table 4.

**Carbon utilization.** The ability of the culture to grow on carbon compounds was determined in the synthetic medium of Pridham and Gottlieb (6) and in their modified medium (8; Tables 5 and 6).

**Temperature.** All cultures grew moderately well to well at temperatures of 18 to 37 C on Bennett's,
Czapek's sucrose, and maltose-triptone agars. Trace to fair vegetative growth occurred in 24 hr at 55 C.

Antibiotic-producing properties. The culture produces the antibiotic scopafungin (U-29,479).

Source. Soil.

Type culture. Streptomyces hygroscopicus CBS, UC 2317.

Variety nova. Streptomyces hygroscopicus var. enhygrus UC 2397, NRRL 3664.

S. hygroscopicus var. enhygrus is a new soil isolate of the genus Streptomyces which has been found to differ in certain characteristics from the type culture S. hygroscopicus CBS, notably in its production of the antibiotic scopafungin, and from the type culture S. endus NRRL 2339 (in its cultural characteristics). S. endus has been shown to have the main characteristics of the hygroscopicus type (2, 9). Minor distinguishing characteristics of the new variety may be noted by referring to the tables.

The new soil isolate, which has been compared to and differentiated from the type cultures S. hygroscopicus and S. endus, is designated S. hygroscopicus var. enhygrus var. nova.

Fermentation studies. A typical fermentation pattern at 28 C is shown in Table 7. The new Upjohn strain fermented in the medium described gave yields of greater potency than were obtained with S. endus NRRL 2339. These beers were thus sufficiently potent to permit the successful extraction and crystallization of scopafungin.

Paper chromatography. The paper chromatographic patterns of crystalline scopafungin in six solvent systems is shown in Fig. 1.

In Vitro spectra. Table 8 shows the in vitro antibacterial spectrum. Inhibition of gram-positive microorganisms occurred at concentrations from 4 to 31 μg/ml. Scopafungin inhibited a wide spectrum of human pathogenic fungi at concentrations of 0.1 to 10 μg/ml (Table 9).

ACKNOWLEDGMENTS

The authors express their appreciation to various members of The Upjohn Co. who contributed to this work. In particular, we thank John A. Fox for the paper chromatography and Walter T. Sokolksi for the standard curve assays.
| Medium | Determination<sup>a</sup> | *S. hygroscopicus var. enhygrus UC 2397* | *S. hygroscopicus CBS, UC 2317* | *S. endus NRRL 2339, UC 2020* |
|--------|--------------------------|------------------------------------------|---------------------------------|--------------------------------|
| Agar   | S                        | Trace white                             | Very slight trace white         | Pale gray white                |
|        | R                        | Yellow-tan                              | Yellow-tan                      | Yellow-tan                     |
|        | P                        | Melanin negative                        | Melanin negative                | Melanin negative               |
| Calcium malate | S            | Trace white                              | Trace white                     | Trace white                    |
|        | R                        | Colorless                               | Colorless                       | Colorless                      |
|        | P                        | None                                    | None                            | None                           |
|        | O                        | Malate not solubilized                  | Malate not solubilized          | Malate not solubilized         |
| Glucose asparagine | S        | Gray-white                              | Gray-white                      | Pale gray white                |
|        | R                        | Cream gray                              | Cream                           | Yellow-pink                    |
|        | P                        | None                                    | None                            | None                           |
|        | O                        | Malate not solubilized                  | Malate not solubilized          | Malate not solubilized         |
| Skim milk | S            | Yellow-pink-white                        | Gray-pink-white                 | Yellow-pink                    |
|        | R                        | Yellow-pink-tan                         | Yellow-pink-tan                 | Yellow-pink                    |
|        | P                        | Yellow-pink                             | Yellow-pink                     | None                           |
|        | O                        | Casein solubilized around growth        | Casein solubilized around growth | Casein solubilized             |
| Tyrosine | S                       | Gray                                     | Gray                            | Gray                           |
|        | R                        | Red-tan                                 | Red-tan                         | Red-tan                        |
|        | P                        | Red-tan                                 | Red-tan                         | Red-tan                        |
| Xanthine | S                      | Tyrosine solubilized                    | Tyrosine solubilized            | Tyrosine solubilized           |
|        | R                        | Pale yellow                             | Pale yellow                     | Pale yellow                    |
|        | P                        | None                                    | None                            | None                           |
|        | O                        | Xanthine not solubilized                | Xanthine not solubilized        | Xanthine not solubilized       |
| Yeast extract-malt extract | S        | Cream-gray-white with moist black patches | Cream-gray-white with moist black patches | Gray-brown                     |
|        | R                        | Yellow-olive                            | Yellow-olive                    | None                           |
|        | P                        | Gray                                    | Gray                            | Gray                           |
|        | S                        | Gray                                    | Gray                            | Gray                           |
|        | R                        | Gray                                    | Gray                            | Gray                           |
|        | P                        | None                                    | None                            | None                           |
|        | O                        | Starch hydrolyzed                       | Starch hydrolyzed               | Starch hydrolyzed              |
| Nutrient starch | S        | White                                   | White                            | White                          |
|        | R                        | Cream                                   | Cream                           | Cream                          |
|        | P                        | Pale yellow                             | Pale yellow                     | Pale yellow                    |
|        | O                        | Starch hydrolyzed                       | Starch hydrolyzed               | Starch hydrolyzed              |
| Sabouroud dextrose | S        | White                                   | White                            | White                          |
|        | R                        | Yellow-tan-orange                       | Yellow-tan-orange               | Yellow                          |
|        | P                        | None                                    | None                            | Yellow                          |
|        | S                        | Heavy gray-white                        | Heavy gray-white                | Heavy gray-white               |
|        | R                        | Yellow                                  | Yellow                          | Yellow                          |
|        | P                        | None                                    | None                            | None                           |
|        | S                        | Heavy gray                              | Heavy gray                      | Heavy gray                     |
| Bennett | R                        | Gray                                    | Gray                            | Trace gray                     |
|        | P                        | None                                    | None                            | Trace gray                     |
|        | S                        | Gray                                    | Gray                            | Trace gray                     |
|        | R                        | Yellow                                  | Yellow                          | Trace gray                     |
|        | P                        | None                                    | None                            | Trace gray                     |
|        | S                        | Maltose-tryptone                        | Maltose-tryptone                | Maltose-tryptone               |
|        | R                        | Yellow                                  | Yellow                          | Yellow                          |
|        | P                        | None                                    | None                            | None                           |
|        | S                        | Peptone-yeast extract (ISP-6)            | Peptone-yeast extract (ISP-6)    | Peptone-yeast extract (ISP-6)   |
|        | R                        | Yellow                                  | Yellow                          | Yellow                          |
|        | P                        | Pale yellow                             | Pale yellow                     | Pale yellow                    |
|        | S                        | Tyrosine (ISP-7)                         | Tyrosine (ISP-7)                | Tyrosine (ISP-7)               |
|        | R                        | Tan                                     | Tan                             | Tyrosine (ISP-7)               |
|        | P                        | Pale pink becoming pink-tan             | Pale pink becoming pink-tan     | Pale pink becoming pink-tan    |

<sup>a</sup> Determination: S = sensitive, R = resistant, P = partial, O = other.
TABLE 4. continued

| Medium          | Determination                                      | S. hygroscopicus var. enhygrus UC 2397 | S. hygroscopicus CBS, UC 2317 | S. endus NRRL 2339, UC 2020 |
|-----------------|---------------------------------------------------|---------------------------------------|--------------------------------|-----------------------------|
| Gelatin         |                                                   |                                       |                                |                             |
| Plain           | P Tan, 1/4                                        | Tan, 1/4                              | Tan, 1/4                        | Tan, 1/4                     |
| Nutrient        | O Liquefaction, 1/2                               | Liquefaction, 1/2                     | Liquefaction, 1/2               | Liquefaction, 1/2            |
| Broth           |                                                   |                                       |                                |                             |
| Synthetic nitrate| S No surface growth                               | Gray aerial growth on surface pellicle| White aerial growth on surface pellicle|                             |
| Nutrient nitrate| S No surface growth                               | Gray aerial growth on surface pellicle| Pale pink-yellow                |                             |
| Litmus milk     | P Compact to flocculent growth at base Nitrate not reduced to nitrite | White aerial growth on surface pellicle| White aerial growth on surface pellicle|                             |
|                 | O Peptonization complete, pH 6.5                  | Peptonization complete, pH 6.5        | Peptonization complete, pH 6.5  |                             |

* S, surface; R, reverse; P, pigment; O, other characteristics.

**TABLE 5. Utilization of carbon compounds in the synthetic medium of Pridham and Gottlieb (6)**

|                  | S. hygroscopicus var. enhygrus UC 2397 | S. hygroscopicus CBS, UC 2317 | S. endus NRRL 2339, UC 2020 |
|------------------|---------------------------------------|-------------------------------|-----------------------------|
| Control          | -                                     | -                             | -                           |
| D-Xylose         | +                                     | +                             | +                           |
| L-Arabinose      | +                                     | +                             | +                           |
| Rhamnose         | +                                     | +                             | +                           |
| D-Fructose       | +                                     | +                             | +                           |
| D-Galactose      | +                                     | +                             | +                           |
| D-Glucose        | +                                     | +                             | +                           |
| D-Mannose        | +                                     | +                             | +                           |
| Maltose          | +                                     | +                             | +                           |
| Sucrose          | (+)                                   | (+)                           | (+)                         |
| Lactose          | +                                     | +                             | +                           |
| Cellulose        | +                                     | +                             | +                           |
| Raffinose        | +                                     | +                             | +                           |
| Dextrin          | +                                     | +                             | +                           |
| Inulin           | -                                     | +                             | +                           |
| Soluble starch   | +                                     | +                             | +                           |
| Glycerol         | +                                     | +                             | +                           |

* Symbols: +, positive utilization; (+), positive utilization—slight growth; - , slight growth—no utilization; −, no growth.
TABLE 6. Utilization of carbon compounds in the modified medium of Pridham and Gottlieb (8)*

| Compound          | S. hygroscopicus var. enhydris UC 2397 | S. hygroscopicus CBS, UC 2317 | S. endus NRRL 2339, UC 2020 |
|-------------------|----------------------------------------|-----------------------------|-----------------------------|
| **Negative control (basal agar)** | Slight growth | Slight growth | Slight growth |
| t-Arabinose       | +           | +             | +                        |
| Sucrose           | +           | ++            | +                         |
| D-Xylose          | +           | +             | ++                        |
| Inositol          | +           | ++            | +                         |
| D-Mannitol        | +           | ++            | +                         |
| D-Fructose        | +           | ++            | +                         |
| Rhamnose          | +           | ++            | +                         |
| Raffinose         | +           | ++            | +                         |
| Cellulose         | +           | +             | -                         |

* Symbols: -, growth similar to or less than growth on basal medium without carbon compound; ++, vegetative growth equal to or more than with glucose; +, vegetative growth significantly better than on basal medium without carbon compound, but somewhat better than with glucose; +, double growth only slightly better than on basal medium without carbon compound and significantly less than with glucose.

TABLE 7. Production of scopafungin and pH patterns in fermentations of Streptomyces hygroscopicus var. enhydris

| Age (hr) | pH  | Scopafungin (µg/ml) | pH  | Scopafungin (µg/ml) |
|----------|-----|---------------------|-----|---------------------|
| 0        | 7.0 | 0                   | 6.9 | 0                   |
| 48       | 7.5 | 1,400               | 7.0 | 1,125               |
| 72       | 7.5 | 2,350               | 7.6 | 1,500               |
| 96       | 7.5 | 3,050               | 7.6 | 2,390               |
| 120      | 7.5 | 4,050               | 7.0 | 3,390               |
| 144      | 7.3 | >5,000              | 7.2 | 4,125               |

* Total antifungal activity expressed as scopafungin.

LITERATURE CITED

1. Dietz, A. 1967. Streptomyces stefisburgensis sp. n. J. Bacteriol. 94:2022-2026.
2. Dietz, A., and J. Mathews. 1962. Taxonomy by carbon replication. I. An examination of Streptomyces hygroscopicus. Appl. Microbiol. 10:258-263.
3. Dietz, A., and J. Mathews. 1968. Taxonomy by carbon replication. II. An examination of eight additional cultures of Streptomyces hygroscopicus. Appl. Microbiol. 16:935-941.
4. Jacobson, E. W. C. Granville, and C. E. Foss. 1948. Color harmony manual, 3rd ed. Container Corp. of America, Chicago.
5. Kelly, K. L., and D. B. Judd. 1955. The ISCC-NBS method of designating colors and a dictionary of color names. U.S. Department of Commerce Circular 553.
6. Pridham, T. G., and D. Gottlieb. 1948. The utilization of carbon compounds by some Actinomycetaceae as an aid for species determination. J. Bacteriol. 56:107-114.
7. Pridham, T. G., C. W. Hesseltine, and R. G. Benedict. 1958.

TABLE 8. Antibacterial spectrum of scopafungin in Brain Heart Infusion broth

| Organism                                | Minimal inhibitory concn (µg/ml) |
|-----------------------------------------|----------------------------------|
| Staphylococcus aureus UC-76             | 8                                |
| S. aureus UC-552                       | 8                                |
| Streptococcus hemolyticus UC-152        | 31                               |
| S. faecalis UC-3235                    | 31                               |
| Escherichia coli UC-51                  | >500                             |
| Proteus vulgaris UC-93                  | >500                             |
| Klebsiella pneumoniae UC-57             | >500                             |
| Salmonella schottmuelleri UC-126        | >500                             |
| Pseudomonas aeruginosa UC-95            | >500                             |
| Bacillus subtilis UC-564                | 4                                |
| Diplococcus pneumoniae UC-41           | 16                               |

TABLE 9. Antifungal spectrum of scopafungin in Whitten's agar medium

| Organism                                | Minimal inhibitory concn (µg/ml) |
|-----------------------------------------|----------------------------------|
| Nocardia asteroides UC-2052             | 10                               |
| Blastomyces dermatitidis UC-1911        | 1                                |
| Coccidioides immitis UC-1119            | 1                                |
| Geotrichum sp. UC-1207                  | 1                                |
| Hormodendrum compactum UC-1222         | 1                                |
| Phialophora verrucosa UC-1807           | 1                                |
| Cryptococcus neoformans UC-1139         | 1                                |
| Histoplasma capsulatum UC-1220          | 0.1                              |
| Sporotrichum schenckii UC-1364          | 10                               |
| Monaspores asporum spinosus UC-1248     | 10                               |
| Trichophyton rubrum UC-1458             | 1                                |
| T. interdigitale UC-1399                | 1                                |
| Candida albicans Ab UC-1077             | 10                               |
| T. violaceum UC-1459                    | 10                               |
| T. mentagrophytes UC-4797               | 1                                |
| Microsporum canis UC-1439               | 10                               |
| T. astroidis UC-4775                    | 10                               |
| T. mentagrophytes UC-4860               | 10                               |

* Tested at concentrations of 100, 10, 1, and 0.1 µg/ml.