in a mixture of 1 ml. of acetic anhydride and 0.5 ml. of dry pyridine, heating the solution to boiling and allowing it to stand overnight. The excess acetic anhydride was decomposed with ice and the crystalline product recrystallized from methanol. The acetate formed tiny, colorless, stout needles, m.p. 163–164° (reported[1b] 164–165°).

Anal. Calcd. for $C_{14}H_{20}O_4$: C, 66.64; H, 7.91. Calcd. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84. Found: C, 66.24, 66.11; H, 7.61, 7.88.

The oxime (VII) was prepared by refluxing for one hour a solution of equal weights of IV, hydroxylamine hydrochloride and sodium acetate in 50% aqueous ethanol. The addition of water and cooling in ice caused the oxime to crystallize in tiny, colorless prisms, m.p. 192–193°. Mixed with IV, the m.p. was 171–175°.

Anal. Calcd. for $C_{12}H_{19}O_3N$: C, 63.96; H, 8.51. Calcd. for $C_{15}H_{23}O_4N$: C, 64.03; H, 8.24. Found: C, 63.65; H, 8.01.

The ultraviolet absorption spectrum of the oxime was substantially the same as that of IV, λ_{max} 239 mμ.

LOS ANGELES, CALIFORNIA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Chartreusin, a New Antibiotic Produced by *Streptomyces chartreusis*, a New Species

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RECEIVED APRIL 22, 1953

Chartreusin is a new antibiotic obtained from *Streptomyces chartreusis*. Analysis of the crystalline antibiotic indicates it to be a weak acid with a formula approximating $C_{18}H_{18}O_8 \cdot 2H_2O$. The ultraviolet and infrared absorption spectra and an antibacterial spectrum are given for chartreusin.

Introduction

A new antibiotic has been obtained from culture filtrates and also from the mycelial mat of two hitherto undescribed *Streptomyces* sp. isolated from the soil. *Streptomyces chartreusis* was isolated from an African soil while another *Streptomyces* sp. which produces this antibiotic was obtained from a Michigan soil. The generic name, chartreusin, has been assigned to this antibiotic because of the characteristic color of the crystalline material.

Chartreusin has been crystallized from concentrates of the culture filtrates as greenish-yellow crystals. The sodium salt also has been crystallized. Elemental analyses and molecular weight determination indicate an empirical formula of $C_{18}H_{18}O_8 \cdot 2H_2O$. At room temperature, the antibiotic is stable for several hours over the range of *p*H 2 to *p*H 10. However, prolonged heating at *p*H 2 or 10 destroys the antibiotic activity.

The ultraviolet and visible spectra, Fig. 1, and the infrared spectrum, Fig. 2, afford further characterization of the antibiotic. In general appearance the ultraviolet spectrum resembles that of substituted 1,2-naphthoquinones.[1,2]

Chartreusin is active against certain Gram-positive organisms and mycobacteria. It also is active against the *Micrococcus pyogenes* v. *aureus* phage.[3]

Table I shows its antibacterial properties.

The acute LD_0 subcutaneously in mice for chartreusin is 2500 mg. per kg.; however, the sodium salt has an acute LD_{50} intravenously in mice of 250 mg. per kg. In chronic studies the antibiotic seems to have a cumulative toxicity, particularly when the sodium salt is used.

Acknowledgments.—The authors are grateful to Dr. J. L. Johnson for the infrared spectrum, Mr. L. Scholten for the ultraviolet studies, Mr. W. A. Struck for the microanalytical data and Mr. O. F. Swoap for the toxicity studies. We wish also to acknowledge the assistance of Dr. H. A. Nelson and Mr. W. H. DeVries for preparation of material.

TABLE I

CONCENTRATION OF CHARTREUSIN REQUIRED TO INHIBIT GROWTH OF MICROÖRGANISMS IN NUTRIENT BROTH

Organism	Mcg./ml.
Bacillus subtilis	1.25
Bacillus subtilis (M.R.-1280)[a]	1.00
Salmonella typhosa	>30
Escherichia coli	>30
Salmonella gallinarum	>30
Salmonella schottmuelleri	>30
Brucella bronchiseptica	>25
Proteus vulgaris	>30
Micrococcus pyogenes v. *aureus*	5.0
Mycobacterium tuberculosis (607)	1.7
Mycobacterium tuberculosis (H37Rv)	2.0

[a] Streptomycin resistant.

Experimental

Assay.—Chartreusin was assayed by the paper-disc method outlined for the assay of fumagillin.[3,4] Fumagillin was used as the assay standard for the isolation work. A unit of activity is defined as being equivalent to one microgram of fumagillin.

Identification of Culture.—This culture which we have named *Streptomyces chartreusis* could not be identified by following the key in Bergey's Manual of Determinative Bacteriology.[5]

The culture is a mesophilic saprophyte with characteristic powdery blue-gray to blue-green spores produced on various media. The sporulating hyphae formed open spirals on some media. White filamentous aerial mycelia were produced, approximately one micron in diameter.

Fermentation.—The organism, *Streptomyces chartreusis*, is carried in culture on agar slants. The surface growth was used to inoculate shaken flasks (500-ml. erlenmeyer flasks each containing 100 ml. of culture broth). The flasks, after about three days of fermentation, were used to inoculate 5-gallon fermenters. These fermenters were

(1) A. K. Macbeth, J. R. Price and F. L. Winzor, *J. Chem. Soc.*, 325 (1935).

(2) R. G. Cooke, A. K. Macbeth and F. L. Winzor, *ibid.*, 878 (1939).

(3) F. R. Hanson and T. E. Eble, *J. Bact.*, **58**, 527 (1949).

(4) F. R. Hanson and T. E. Eble, *Antibiotics and Chemotherapy*, **1**, 54 (1951).

(5) R. S. Breed, E. G. D. Murray and A. P. Hitchens, "Bergey's Manual of Determinative Bacteriology," 6th Ed., The Williams and Wilkins Company, Baltimore, Md., 1948, pp. 929–933.

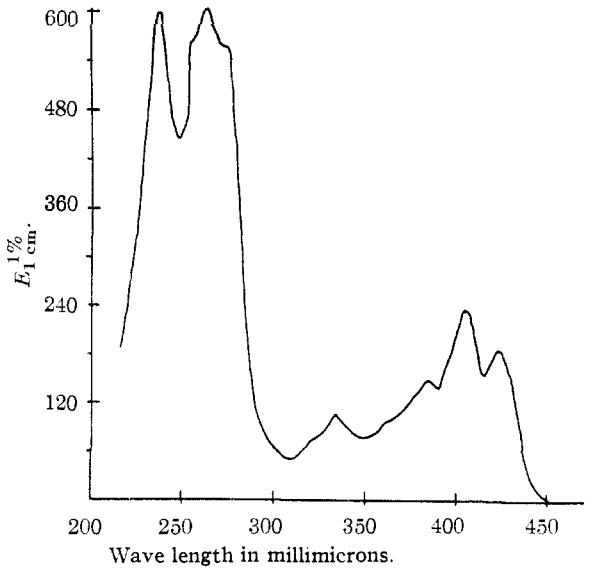

Fig. 1.—Ultraviolet absorption spectrum of chartreusin in 95% ethanol (Cary Recording Spectrophotometer).

Isolation of Chartreusin from Fermentation Broths.—Two hundred liters of a typical culture broth assaying 21 units per ml. was filtered on a plate and frame filter using 2% Dicalite 4200.[6] A considerable amount of the antibiotic remains associated with the mycelial cake, and it can be obtained by extracting with 80% acetone. Extraction of the filtered beer with chloroform yielded the first crystalline preparation. The filtered beer, 200 liters assaying 3.6 units per ml., was extracted with three 10-liter portions of chloroform. The extracts were combined, evaporated *in vacuo* to dryness and dissolved in minimum volume acetone to yield pale yellow-green crystals. In ultraviolet light these crystals exhibited a characteristic fluorescence. Recrystallization of the crude product from acetone–water afforded 1.18 g. assaying 150 units per mg. The yield from the filtered beer to the recrystallized product was 25%. An analytical sample was recrystallized from acetone, m.p. 180°.

Anal. Calcd. for $C_{18}H_{18}O_8 \cdot 2H_2O$: C, 54.27; H, 5.57; C–$CH_3$, 7.55; H_2O, 9.04. Found: C, 54.53; H, 5.50; C–CH_3, 6.22; wt. loss on drying, 8.91. *Anal.* Calcd. for $C_{18}H_{18}O_8$ (after drying to constant weight at 56°): C, 59.66; H, 5.01, mol. wt., 362.32. Found: C, 59.89; H, 5.19, mol. wt. (Rast camphor), 357.

Preparation of the Sodium Salt.—Three hundred forty-three milligrams of chartreusin was suspended in 5 ml. of water and 2.5 ml. of acetone and titrated to *p*H 9.96 with sodium hydroxide. Filtration through sintered glass afforded a clear, dark, amber solution which was freeze-dried.

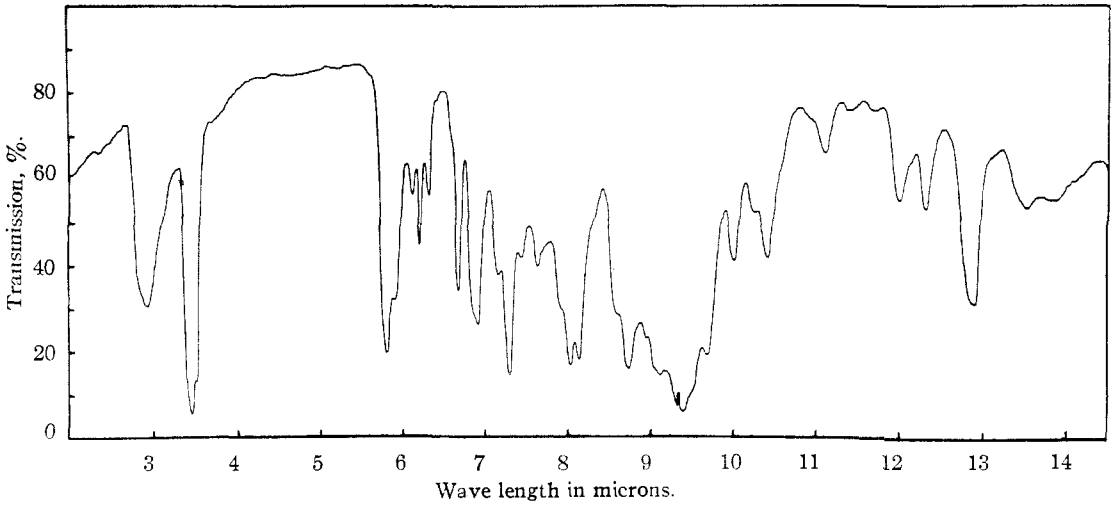

Fig. 2.—Infrared absorption spectrum of chartreusin in Petrolatum mull (Perkin–Elmer model 21 spectrophotometer).

used to seed the 100-gallon fermenters. The fermentation tanks were maintained at 27° for approximately four days before harvesting. These tanks were aerated with sterile air at a flow rate of 0.5 to 2 volumes of air per volume of broth per minute.

The fermentation medium has the following composition: glucose 25 g., brewer's yeast 2.5 g., ammonium sulfate 5 g., calcium carbonate 8 g., sodium chloride 4 g., potassium dihydrogen phosphate 0.4 g., soybean meal 7 g., and water to make 1000 ml. The fermentation broths were harvested at approximately 90 hours.

The golden-yellow product weighed 310 mg. The sodium salt crystallized from water as small golden-colored needles or plates. This salt is soluble to the extent of 20 mg. per ml. or more in water at *p*H 9.5; however, exposure to carbon dioxide or acidification to below *p*H 9 re-precipitates the acid.

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(6) Dicalite 4200 is a diatomaceous earth supplied by the Great Lakes Carbon Corporation.