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U-21,963, a New Antibiotic

II. Isolation and Characterization

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ABSTRACT

MEYER, CURTIS E. (The Upjohn Co., Kalamazoo, Mich.). U-21,963, a new antibiotic. II. Isolation and characterization. Appl. Microbiol. **14**:511–512. 1966.—The isolation and characterization of antibiotic U-21,963 are discussed. This compound is a highly unsaturated monobasic acid with the molecular formula $C_9H_7NO_3$. The molecular weight is 177. It is dextrorotatory, $[\alpha]_D = +138°$, and has a pK_a of 5.1. The ultraviolet absorption spectrum, which showed a maximum at 223 mμ (ϵ = 15,115), indicates unsaturation α-β to the carboxyl group, and the infrared spectrum suggests the presence of an acetylenic group. Explosive decomposition of U-21,963 at 97 C conforms with the latter. U-21,963 is relatively insoluble in water, but readily soluble in ethyl alcohol, acetone, and halogenated hydrocarbons.

A new antibiotic, U-21,963, whose fermentation and biological properties are described by Pyke and Dietz (2), has been obtained from fermentation of *Trichoderma viride* strain UC 4785. Its isolation and chemical and physical properties are described in this paper.

ISOLATION AND PURIFICATION

Antibiotic U-21,963 was isolated by the following process. The filtrate from a fermentation broth was chilled to 5 C; this temperature was subsequently maintained. The filtrate was extracted at *p*H 5.2 with ethyl acetate, the antibiotic was transferred first to water at *p*H 7.0, and then to methylene chloride at *p*H 4.5. The methylene chloride extract was dried with anhydrous sodium sulfate, and, after concentration and decolorization with charcoal, 5 volumes of cold hexane was added. The antibiotic promptly crystallized in the form of needles.

Although the crystals were at first colorless, they rapidly turned brown, due, apparently, to surface polymerization. Further change appeared to be inhibited by the surface coating of the pigment.

CHARACTERIZATION

The ultraviolet spectrum of U-21,963 (Fig. 1) obtained in either alcohol or 0.01 N alcoholic sulfuric acid solution showed a maximum at 223 mμ, with molar extinction coefficients of 15,115 and 14,900, respectively. The maximum in 0.01 N alcoholic potassium hydroxide solution shifted to below 200 mμ. Both the absorption in this region and the magnitude of the ϵ values are characteristic of compounds with the general formula X—C≡C—C≡C—Y (1).

The infrared absorption spectrum (Fig. 2) has a strong band at 2,120 cm^{-1}, attributable to an acetylenic group; a "medium" band at 2,680 cm^{-1}, indicative of a carboxylic OH; a strong band at 1,685 cm^{-1}, indicating α-β conjugation to a carboxyl group and supporting the ultraviolet data; and strong bands at 1,662, 1,639 and 1,609 cm^{-1}, characteristic of C=O, C=C, or C=N groups, or all three.

A solution of freshly crystallized antibiotic U-21,963 in 50% ethyl alcohol initially had $[\alpha]_D^{25} = +138.6°$ (c, 0.44). In 0.1 N alcoholic sodium hydroxide solution, the specific rotation rapidly increased with considerable darkening so that observations could not be made after 25 min. On the other hand, in 0.1 N alcoholic hydrochloric acid solution, the optical activity decreased rapidly for the first hour and then asymptotically. The data are presented graphically in Fig. 3.

The compound melts with explosive decomposition at 97 C.

EXPERIMENTAL

The broth obtained from a 540-liter fermentation of *T. viride* UC 4785 was filtered at the harvest *p*H of 5.2 after the addition of 3% filter aid. The filtrate was chilled to 5 C, then extracted three times with one-third beer volumes of cold ethyl acetate. The extracts were combined and extracted 3 times with cold water at a *p*H of 7.0.

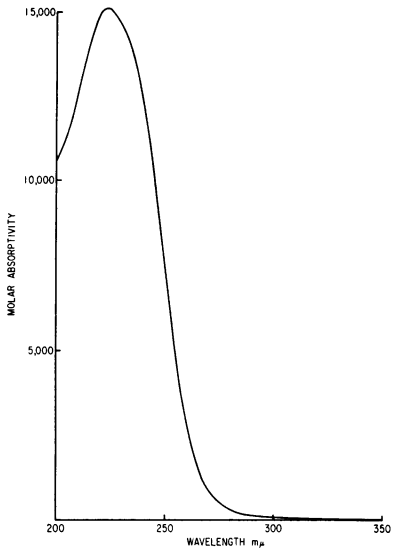

FIG. 1. *Ultraviolet absorption spectrum of antibiotic U-21,963.*

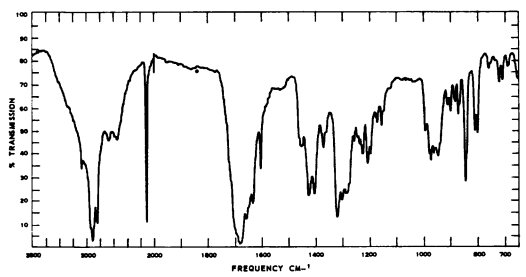

FIG. 2. *Infrared spectrum of antibiotic U-21,963.*

The combined aqueous extracts, amounting to 220 liters, were concentrated at reduced pressure, and at a temperature not exceeding 35 C, to 20 liters. Eighteen liters of the concentrate was freed from fatty materials by extraction with commercial hexane, then extracted at *p*H 4.5 with 40 liters of cold methylene chloride in four equal portions. The extracts were combined and rinsed with 250 ml of water, dried with anhydrous sodium sulfate, and concentrated to 2.8 liters at 20 C. Of this concentrate, 700 ml was decolorized with 3 g of charcoal, reduced to about 300 ml under diminished pressure, and chilled, and 1,500 ml of cold commercial hexane was added. White crystals formed immediately; after 10 min, they were filtered. Traces of solvent were removed in a vacuum desiccator, affording 3.1 g of material. The crystals rapidly

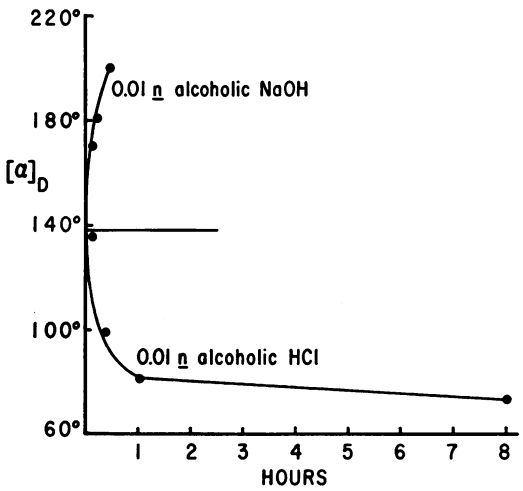

FIG. 3. *Change in specific rotation of U-21,963 in 0.01 N NaOH and HCl.*

became brown, but gave satisfactory analyses and consistent biological values in a *Sarcina lutea* assay over a period of several days.

Analyses: found (on 2 different preparations): C, 60.18, 60.17; H, 4.09, 3.98; O, 26.78; N, 7.56, 8.12. $C_9H_7NO_3$ requires: C, 61.0, H, 3.96; O, 27.15; N, 7.91; mol wt = 177. The molecular weight determined by mass spectroscopy was found to be 177.

U-21,963 potassium salt. The potassium salt was prepared by adding 260 mg of potassium bicarbonate in 1 ml of water to 500 mg of the acid dissolved in 3 ml of absolute alcohol. The addition of 15 ml of acetone caused crystallization of 430 mg of the dried salt.

Analysis: $C_9H_6NO_3K$; calculated: C, 50.20; H, 2.78; N, 6.51; K, 18.16; found: C, 49.72; H, 2.52; N, 6.15; K, 17.79.

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