XANTHOCILLIN, A METABOLITE OF *EUPENICILLIUM* EGYPTIACUM NRRL 1022

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During a screening study of the members of the genus *Eupenicillium* for secondary metabolites, it was found that *Eupenicillium egyptiacum* (st. *imperfect* = *Penicillium egyptiacum*) NRRL 1022 produced xanthocillin on modified Czapek Dox medium. The antibiotic xanthocillin was first isolated from *P. notatum* Westling by Rothe in 1950 (1). This *Penicillium* is a member of the *P. chrysogenum* series, which regularly occurs in soil and is commonly found on decaying vegetation and on a wide variety of foods (2). Xanthocillin is also produced by *Aspergillus* sp. (3), *A. chevalieri* (4) and *Dichotomomyces albus* (5). Rothe (1) has demonstrated xanthocillin antibiotic properties, and Townsend *et al.* (4) documented its hepatotoxicity to animals. The molecular structure of this hepatoxic antibiotic is unusual because of its isonitrile group. This report describes the isolation of xanthocillin from *P. egyptiacum* and its identification by spectroscopic methods. Further characterization was made by conversion to its diacetate and oxidation of this product to p-acetoxybenzoic acid.

EXPERIMENTAL

CULTIVATION OF ORGANISM.—*P. egyptiacum* NRRL 1022 most likely represents the strain CBS 244.32 *P. egyptiacum* van Beyma.

The stock culture of the fungus was maintained on YM agar slants (1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt exextract and 2% agar). A steam-sterilized liquid medium, consisting of Staley corn steep liquor (10 ml), $NaNO_3$ (3.0 g), K_2HPO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KCl (0.5 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), distilled H_2O (1 liter) [pH adjusted to 7 with 1N NaOH] contained in a Fernbach flask, was inoculated with 1 ml of a cell suspension prepared from a 7-day-old YM slant culture and 5 ml of sterile distilled water. The inoculated medium was incubated at 25° as stationary cultures for 30 days.

ISOLATION OF XANTHOCILLIN.—The mycelium, which fragmented into small pieces after the third week, was filtered with the aid of Celite from the liquid medium. The Celite-mycelium mixture (from 1 liter of culture broth) was dried at room temperature overnight and then was extracted with acetone by refluxing for 2 hr. On evaporation of the acetone, 1.1 g of a brown solid A remained. Ethyl acetate extraction of solid A (600 mg) at room temperature gave 200 mg of solid B, which crystallized from acetone in the form of fine needles (C) (100 mg).

IDENTIFICATION OF XANTHOCILLIN.—The yellow needles (C) decomposed at 205°, and elementary analyses corresponded to a $C_{18}H_{12}N_2O_2$ (Anal. Calcd. C, 74.9; H, 4.3; N, 9.7; NW 288. Found: C, 74.6; H, 4.2; N, 9.5; parent ion *m/e* 288); ir, νmax (KBr) 3300, 2110, 1600, 1505, 1435 cm^{-1}. The ir was superimposable on the spectrum for xanthocillin published by Hagedorn and Tonjes (6). The nmr spectrum [$(CD)_3CO$, 100 MHz] showed signals at δ 7.8 (d, 4H, J=9 Hz), 6.96 (d, 4H, J=9 Hz) and δ 3.86 (s, 2H). Ten milligrams of needles (C) was converted to its diacetate in pyridine (0.1 ml) and acetic anhydride (0.1 ml) at room temperature. The diacetate crystallized out of the reaction medium and gave 11 mg of fine needles, which decomposed at 200° [lit. 200° (6)]. Anal. Calcd. for $C_{22}H_{18}N_2O_4$: C, 70.94; H, 4.30; N, 7.50. Found: C, 71.0; H, 4.25; N, 7.6. High resolution mass spectrometry showed a molecular ion at *m/e* 372 with a composition of $C_{22}H_{18}N_2O_4$. The primary fragmentation was two losses of 42 (CH_2CO) to give ions at 330 and 288 *m/e* which was in accord for loss of two acetate units. The ir νmax (KBr) 2115, 1760, 1598, 1505, 1415, 1365, 1210, 1190, 1168, 1015, 1008, 910, 890, 835, 825, 810 (sh), 618 cm^{-1} and the nmr ($CDCl_3$, 100 MHz) signals

at δ 2.22 (6H, 2 O-C-CH_3), δ 7.8 (d, 4H, J=

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9 Hz), δ 7.1 (d, 4H, J=9 Hz) and δ 3.86 (s, 2H) are also in agreement for the diacetate of xanthocillin.

The diacetate (10 mg), oxidized with $KMnO_4$ in acetone at room temperature, gave 8 mg of *p*-acetoxybenzoic acid. The identity was established by ir, mixture melting point, and mass spectrum. The formation of the diacetate of xanthocillin, its oxidation to p-acetoxybenzoic acid, and comparison of the ir of xanthocillin with the published spectrum, as well as nmr data, show the compound produced by *P. egyptiacum* to be identical with xanthocillin.

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