PRODUCTION OF TROPOLONE BY A *PSEUDOMONAS*

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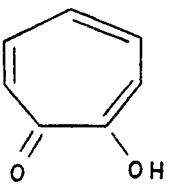
ABSTRACT.—A bacterium with strong antimicrobial activity was characterized as a member of the genus *Pseudomonas*. The mass spectroscopy spectrum of the crystalline antibiotic obtained from the Pseudomonas filtrate was identical to that of tropolone (**1**), as were the uv, ir, pmr and cmr spectra.

Dewar (2) reported the structure of stipitatic acid, a metabolite of *Penicillium stipitatum*. He suggested that stipitatic acid and colchicine, a natural product of certain Liliaceae, were members of a new system of "nonbenzenoid aromatic compounds." He then predicted the parent cycloheptatrienolone and proposed the term tropolone for it. Several other tropolones are produced by fungi (3) besides Dewar's stipitatic acid. Other tropolones that have been reported to occur in nature are those present in western red cedar (5) of which the isopropyl tropolone, β-thujaplicin (4-isopropyltropolone), is probably the best known (7). Such tropolones presumably are responsible for the durability of red cedar.

A bacterium (*Pseudomonas* ATCC 31099) with strong antimicrobial activity was found among colonies of *Helminthosporium cynodontis* Marig. isolated from bermuda grass (*Cynodon dactylon* (L.) Pers.). This report is concerned with the identification of tropolone as the antibiotic agent produced by the *Pseudomonas*. Tropolone, 2-hydroxy-2,4,6-cycloheptatriene-1-one (**1**), seems not to have been reported from nature (5, 6), but the antibacterial activity of tropolone has been reported (6).



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EXPERIMENTAL AND RESULTS

PRODUCTION OF ANTIBIOTIC.—The *Pseudomonas* and its antimicrobial activity were first observed on potato dextrose agar (300 g peeled, diced potatoes, 23 g dextrose, and 15 g Difco agar in 2 liters of distilled water). Production trials of the antibiotic in liquid culture, therefore, were first made in potato dextrose broth (PDB, ingredients were the same as above except that the agar was deleted). The *Pseudomonas* was seeded to 600 ml of PDB in 2 liter Erlenmeyer flasks, placed under fluorescent light for 72 hrs and thereafter incubated without added light for 11 days at 22°. Stock cultures of the *Pseudomonas* were maintained by lyophilization or as a suspension in sterile distilled water at 6°.

ISOLATION OF TROPOLONE (**1**).—Eight liters of concentrated (*ca* 4 fold) fermen-

tation filtrate of the *Pseudomonas*, sent from the laboratories of Louisiana State University to the Upjohn Company, were used for the isolation and identification of tropolone as the antibacterial component. After adjustment to pH 3 with 6 *N* HCl, the fermentation concentrate was extracted twice with four liter portions of dichloromethane. These combined extracts contained essentially all the antimicrobial activity as determined by paper disc-agar diffusion assay against *Penicillium oxalicum*. Concentration of these extracts *in vacuo* gave 3.06 g of crude extract solids which was purified by chromatography over a 50 g column (1.9 x 45 cm) of silica gel-60 (E. Merck #7734) prepared in dichloromethane. Dichloromethane containing 2% methanol (v/v) eluted the antibiotic as shown by bioassay. The chromatographic product appeared as a single component, R_f 0.65, by tlc on MN-polygram sil N-HR (Brinkman Instruments, Inc.) developed in dichloromethane-methanol (9:1 v/v) and bioautographed on any of several microorganisms: *Bacillus subtilis*, *Sarcina lutea*, *Saccharomyces pastorianus*. Concentration of the active fractions gave an unweighed dark, gummy solid which resisted further purification by silica gel chromatography.

A portion of the chromatographic product was further purified by sublimation under reduced pressure; a dry ice cooled, cold finger trap was used to collect the colorless sublimate. A portion of this sublimate, when crystallized from aqueous acetone, produced colorless plates, mp 50–51°.

The crystalline antibiotic obtained from the *Pseudomonas* fermentation was identified as tropolone first by mass spectroscopy. A molecular ion at M+122.0372 ($C_7H_6O_2$=122.0368) was obtained which displayed strong fragmentation peaks at 94.0418 (C_6H_6O=94.0418), 66 and 65. The spectrum was identical to that of tropolone (1). Uv, ir, pmr and cmr spectra were either identical to that reported for tropolone or identical to that obtained from a known sample of tropolone.[1]

TABLE 1. Additional characteristics of the organism.

Characteristics	Reaction
Nitrate reduction	+
B-galactosidase	−
Arginine dihydrolase	−
Lysine decarboxylase	−
Ornithine decarboxylase	−
Citrate utilization	−
H_2S production	−
Urease production	−
Phenylalanine deaminase	−
Indole production	−
Voges-Proskauer (acetoin)	−
Malonate utilization	−
Gelatin hydrolysis	−
Esculin hydrolysis	−

THE BACTERIUM.—The bacterium is a Gram negative, straight rod, and is motile with approximately six polar flagella. A bright red-to-orange pigment is produced in potato dextrose broth standing culture under fluorescent light. Its metabolism is respiratory; it does not produce acid from the carbohydrates glucose, rhamnose, sucrose, melibiose, amygdalin or arabinose, or from the alcohols mannitol, inositol, or sorbitol (4). It is catalase and oxidase positive. These

[1]Aldrich Chemical Company, #T8, 970-2.

characteristics warrant its placement in the genus *Pseudomonas*. Additional characteristics of the organism were determined either with the Minitek (BBL, Cockeysville, MD) or Path-o-teck (Warner-Chilcott) systems and are given in table 1.

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