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N-Demethylation of Lergotrile by *Streptomyces platensis*

PATRICK J. DAVIS, JOHN C. GLADE, ALICE M. CLARK, AND ROBERT V. SMITH*

Drug Dynamics Institute and Division of Pharmaceutical Chemistry, College of Pharmacy, University of Texas at Austin, Austin, Texas 78712

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Thirty-eight microorganisms were screened for their ability to produce metabolites of the semisynthetic alkaloid, lergotrile. A total of five microorganisms were found to biotransform lergotrile, and *N*-desmethyl lergotrile was detected as the principal metabolite with most organisms. *Streptomyces platensis* (NRRL 2364) appeared to form the metabolite in highest yield, and a preparative-scale conversion was accomplished with a recovered yield of 50%. Structure proof was accomplished with comparative thin-layer chromatography, mixed melting point, mass spectrometry, and remethylation to lergotrile.

Recent interest in lergotrile (Fig. 1, I) stems from its putative dopaminergic activity and inhibition of prolactin secretion. Lergotrile has been tested extensively in treatment of Parkinsonism and shown to provide marked reduction in tremors (8). It has been effective in treating galactorrhea of many etiologies due to its inhibition of prolactin secretion (5). It may also be useful in treating acromegaly (6) and reversing infertility in women (3). However, side effects of orthostatic hypotension, mental change, and abnormal liver function have been observed with its use. The hepatotoxicity is particularly disturbing and has disrupted clinical trials (1). This toxicity may be related to the biotransformation of lergotrile in man and has prompted a study of lergotrile's microbial metabolism. Using the strategy of reference 10, a primary objective is to obtain sizable quantities of metabolites for structural identification and biological testing.

In guinea pigs, lergotrile is converted to desmethyl lergotrile (Fig. 1, II); lergotrile is also hydroxylated at position thirteen, and is subject to hydrolysis of its nitrile function (C. J. Parli and B. Schmidt, Fed. Proc. **34**:813, 1975). The phenolic metabolite 13-hydroxylergotrile has also been found in humans (9). Initial efforts to describe microbial systems that mimic the mammalian metabolism of lergotrile have led to the discovery that *Streptomyces platensis* (NRRL 2364) produces the *N*-demethyl metabolite in high yield. The preparative-scale production of desmethyl lergotrile using *S. platensis* is the subject of this report.

MATERIALS AND METHODS

Materials. Lergotrile mesylate and desmethyl lergotrile were supplied by Eli Lilly Co., Indianapolis, Ind. All solvents and reagents were of analytical reagent grade or better.

Analytical methods. Mass spectra were obtained on a DuPont model 21491 mass spectrometer. Thin-layer chromatography (TLC) was performed using three systems: (i) silica gel (Polygram Sil G/UV 254, 0.25 mm; Brinkman) developed with chloroform methanol-acetone-ammonium hydroxide (28%) (63:7:27:2, vol/vol); R_f values—lergotrile = 0.49, desmethyl lergotrile = 0.32; (ii) aluminum oxide (Polygram Alox N/UV 254, 0.20 mm; Brinkman) developed with toluene-morpholine (18:2, vol/vol); R_f values—lergotrile = 0.57, desmethyl lergotrile = 0.24; (iii) aluminum oxide (same as in ii) developed with acetone; R_f values—lergotrile = 0.46, desmethyl lergotrile = 0.39. Melting point determinations were done with an oil bath apparatus (Thomas Hoover) with a heating rate of 1°C/min, and are corrected.

Screening and fermentation conditions. Based on literature precedence (4, 10), 38 microorganisms (Table 1) were screened for their ability to oxidize and *N*-demethylate lergotrile. Cultures were maintained on either refrigerated (4°C) Sabouraud-maltose (Difco) slants or ATCC medium 5 sporulation agar slants. Cultures were transferred to fresh slants every 4 months to maintain viability. The medium used in all studies consisted of the following: dextrose, 20 g; soybean meal (20 mesh, Capital Feeds, Austin, Tex.), 5 g; yeast extract (Difco), 5 g; NaCl, 5 g; K_2HPO_4, 5 g; distilled water, 1,000 ml; pH adjusted to 7.0 with 5 N HCl. The medium was sterilized in an autoclave at 121°C for 15 min.

The surface growth of a slant of each microorganism was used to inoculate 125-ml Erlenmeyer flasks containing 25 ml of the soybean-dextrose media and stoppered with Dispo-plugs (Scientific Products). These stage 1 flasks were agitated on a rotary shaker (Lab-line Orbit Shaker model 3590) at 250 rpm (2.54-cm stroke) at 25°C for 72 h. After 72 h, 3 ml of stage 1 growth was used to inoculate the stage 2 125-ml Erlenmeyer flasks containing 25 ml of the soybean-dextrose media. These cultures were incubated for 24 h before the addition of 6.25 mg of lergotrile dissolved in 1 ml of water. Samples of 2 ml were drawn at 24 and 72 h, made alkaline with sodium carbonate-sodium bicarbonate buffer (pH 8.5), and extracted with 4 ml of

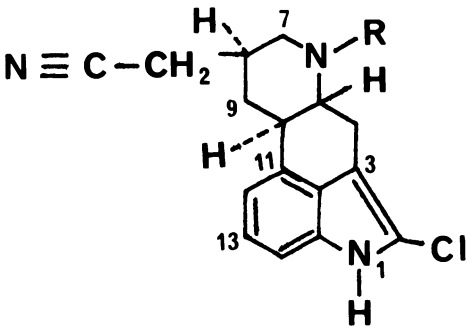

FIG. 1. *Chemical structure of lergotrile (R-CH_3) and desmethyl lergotrile (R-H).*

ethyl acetate. The organic layer was evaporated to dryness under a stream of nitrogen, redissolved in ethyl acetate, and spotted on TLC plates for analysis. Control incubations were conducted with lergotrile in sterilized medium, and autoclaved medium containing organisms was found to possess *N*-dealkylase activity.

Preparative-scale production of desmethyl lergotrile. *S. platensis* (maintained on ATCC medium 5 sporulation agar slants) was grown in a manner similar to the procedure described above but with the following exceptions: 6 ml of the stage 1 culture was used to inoculate each of eight stage 2 250-ml Erlenmeyer flasks containing 50 ml of medium. It was necessary to incubate these stage 2 flasks for 72 h rather than 24 h before substrate addition to obtain adequate growth of *S. platensis*. Lergotrile mesylate (100 mg) in 800 μl of sterile water was distributed evenly among the stage 2 cultures (final concentration, 0.25 mg/ml). After 2 days of incubation, complete conversion of substrate was shown by TLC system iii. The entire fermentation culture was exhaustively extracted by combining 10-ml portions with 10 ml of 0.1 M sodium carbonate/sodium bicarbonate buffer (pH 8.5) and 10 ml of diethyl ether in each of forty, 50-ml screw-capped test tubes (silyated with 2% trimethylsilyl chloride [Pierce] in toluene and dried at 100°C for 1 h). The tubes were rocked on a Lab-Tek Aliquot Mixer (30 oscillations per min) for 30 min. The tubes were centrifuged (1,230 × *g*) for 2 to 3 min, and the ether layers were drawn off and combined. The ether extract was dried with anhydrous Na_2SO_4 and evaporated to dryness in vacuo. The residue (84 mg) was redissolved in 5 ml of methanol and applied to two preparative TLC plates (20 by 20 cm, silica gel PF 254 [Merck], 1.0 mm thickness; activated at 100°C for 1.5 h). Development was carried out in chloroform-methanol-acetone-NH_4OH (28%) (63:7:27:2, vol/vol). The band representing desmethyl lergotrile, located with short-wavelength (254 nm) ultraviolet light, was scraped off, and the metabolite was eluted with acetone. Filtration and evaporation of solvent afforded 36.2 mg of desmethyl lergotrile (pure by TLC) which represents approximately 50% recovery. The product was crystallized from methanol, the mp of this material (221 to 224°C) was identical with authentic desmethyl lergotrile, and there was no depression of mp upon admixture. A mass spectrum (ms) of the product was essentially identical to that of authentic desmethyl lergotrile; ms (m/e, % rel. abund.), 285 (100) (M^+), 245 (8), 217 (12), 188 (31), 178 (21), 154 (25), and 127 (12). Analytical TLC in the three systems showed identical R_f values for the product and authentic desmethyl lergotrile. Both product and authentic desmethyl lergotrile gave an identical blue-color reaction after treatment with Van Urk-Salkowski (modified) reagent (2). A 2-mg portion of metabolite desmethyl lergotrile was remethylated to lergotrile by treatment with formaldehyde and sodium borohydride at room temperature (7), as indicated by TLC in system iii.

TABLE 1. *Microorganisms screened for their ability to biotransform lergotrile*

Microorganism	Source and reference no.[a]
Aspergillus niger	UI X-172
Cunninghamella bainieri	UI-3065
C. blakesleeana	ATCC 8688a
C. blakesleeana	NRRL 1369
C. blakesleeana	UI SC-2138
C. echinulata	ATCC 9244
C. echinulata	NRRL A-11498
C. echinulata	UI 1387
C. echinulata	UI 3655
C. elegans	ATCC 9245
C. elegans	UI 1393
Gliocladium deliquescens	NRRL 1086
Helicostylum piriforme	QM-6945
Microsporum gypseum	ATCC 11395
Mucor mucedo	UI 1605
Pellicularia filamentosa	NRRL 2727
Saccharomyces cerevisiae	NRRL Y-2034
Sepedonium chrysospermum	ATCC 13378
Streptomyces aureofaciens	ATCC 11304
S. flocculus	ATCC 25453
S. griseus	ATCC 10137
S. griseus	NRRL B-599
S. griseus	NRRL 3242
S. griseus	UI L-103
S. griseus	UI-1158
S. lavendulae	UI L-105
S. lincolnensis	ATCC 25466
S. lincolnensis	NRRL 2936
S. paucisporogenes	ATCC 12956
S. platensis	NRRL 2364
S. punipalus	NRRL 3529
S. punipalus	UI 3529
S. rimosus	ATCC 23955
S. rimosus	NRRL 2234
S. rutgersensis	NRRL B-1256
S. scabies	UI SC-1627
Streptomyces species	UI MR-127
S. spectabilis	UI C-632

[a] Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; NRRL, Northern Regional Research Laboratories, Peoria, Ill.; QM, Quartermaster Collection, Mycological Services, Amherst, Mass.; UI, College of Pharmacy, University of Iowa, Iowa City, Iowa.

RESULTS AND DISCUSSION

Thirty-eight microorganisms (actinomycetes, yeasts, and fungi; Table 1) were screened for their ability to metabolize lergotrile mesylate, based on literature precedence of known oxidative transformations (4, 10). Five cultures [*Cunninghamella echinulata* (UI 3655), *Streptomyces rimosus* (ATCC 23955), *Streptomyces platensis* (NRRL 2364), *Streptomyces spectabilis* (UI C-632), and *Streptomyces flocculus* (ATCC 25453)] all produced metabolite desmethyl lergotrile as indicated by TLC. Compound desmethyl lergotrile was not detected when lergotrile was incubated in sterilized medium or autoclaved medium plus organisms found to possess *N*-dealkylase activity. *S. platensis* was used for preparative-scale production since TLC analysis indicated complete, uncomplicated conversion. Desmethyl lergotrile, a known mammalian metabolite, was thus isolated in 50% overall yield, and shown to be identical to authentic desmethyl lergotrile by physical, spectral, and chromatographic comparisons, as well as remethylation to lergotrile. A possible loss in vitality of *S. platensis* required a longer incubation time before substrate addition than we usually observe for optimum growth of *Streptomyces* species. However, this did not seem to decrease the extent of conversion if sufficient time was allowed for incubation.

Since some of the mammalian metabolites of lergotrile are potent dopamine agonists (9), correlating metabolic pathways in microbial and mammalian species offer the potential of large-scale production of more clinically suitable derivatives. Since desmethyl lergotrile is a metabolite in microbes and mammals, it appears feasible that other mammalian metabolites might be made available through continued study. The preparation of desmethyl lergotrile from lergotrile as described in this report provides additional evidence for the utility of the "microbial models" approach in drug metabolism studies (10).

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