

Microbial Transformations of Pergolide to Pergolide Sulfoxide and Pergolide Sulfone

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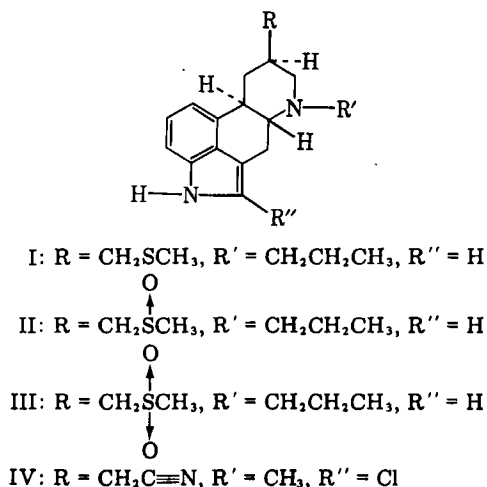
Received February 22, 1982, from the ^{*}Drug Dynamics Institute and [‡]Division of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Texas at Austin, Austin, TX 78712. Accepted for publication June 22, 1982.

Abstract □ Fifty-eight microorganisms were investigated for their ability to effect the biotransformation of the ergoline alkaloid pergolide. A majority of these organisms formed pergolide sulfoxide, and a *Helminthosporium* species was investigated in greater detail since it yielded significant amounts of pergolide sulfoxide. A preparative-scale transformation afforded material which was identified as the sulfoxide based on melting point, spectral, and chromatographic comparison with authentic material as well as its conversion to pergolide by reduction with triphenylphosphine. An analytical high-performance liquid chromatographic determination of the enzymatic *versus* spontaneous air-oxidation of pergolide in growing cultures and controls showed negligible air-oxidation and an ~40% enzymatic conversion of pergolide to the sulfoxide. Several organisms, including *Aspergillus alliaceus* formed a second metabolite, pergolide sulfone, which was identified on the basis of co-chromatographic data.

Keyphrases □ Pergolide—sulfoxide and sulfone metabolites, biotransformation in 58 microorganisms, identification by preparative-scale TLC and high-performance liquid chromatography □ TLC, preparative-scale—of pergolide and its sulfoxide and sulfone metabolites, following microbial transformation □ High-performance liquid chromatography—of pergolide and its sulfoxide and sulfone metabolites, following microbial transformation

The dopaminergic properties of ergoline alkaloids are responsible for their use in the treatment of prolactin-dependent disorders such as galactorrhea and amenorrhea, hyperprolactinemic anovulation, prolactin-dependent breast cancers, and other dopaminergic disorders such as acromegaly and Parkinson's disease (1–8). Ergoline derivatives such as bromocriptine and lergotril are potential inhibitors of prolactin secretion (9, 10), and bromocriptine also may be useful therapeutically in combination with levodopa for the management of “on-off” reactions in parkinsonism (11). At dose levels needed to treat this disease, however, hepatotoxicity has been observed with lergotril (11), which has led to the disruption of clinical trials.

As a result of studies undertaken to define the structural



requirements necessary for prolactin inhibition and to develop more selective dopaminergic agents for the treatment of hyperprolactinemic states and Parkinson's disease (12–14), a semisynthetic ergoline, pergolide (I), was investigated and found to be one of the most potent dopamine agonists *in vitro* and *in vivo* (15–17). Clinical investigations of I in the treatment of galactorrhea and amenorrhea (18) indicated that I was a more potent prolactin inhibitor than previously studied ergolines, and that it had a longer duration of action with more tolerable side effects. Pergolide also has shown marked advantages compared with bromocriptine and lergotril, either alone or in combination with levodopa, in the treatment of Parkinson's disease (19).

Two metabolites of I, pergolide sulfoxide (II) and pergolide sulfone (III), have been reported in mammalian systems¹. Using the rationale of “microbial models of mammalian metabolism” (20, 21), a study was undertaken to identify microorganisms which form the two metabolites found in mammalian species. The present report describes the investigation of 58 microorganisms for their ability to effect the biotransformation of I, the preparative-scale conversion and identification of the metabolite pergolide sulfoxide (II) from *Helminthosporium* species NRRL 4671 cultures, the identification of pergolide sulfone (III) in *Aspergillus alliaceus* UI 315 cultures, and the high-performance liquid chromatographic (HPLC) determination of the enzymatic *versus* spontaneous air-oxidation of I to II from growing cultures and controls.

EXPERIMENTAL

Materials—Pergolide mesylate², pergolide sulfoxide², and lergotril mesylate² (internal standard) were used without further purification. Water for use in the HPLC was deionized and double-distilled in glass; acetonitrile³ was HPLC grade. The mobile phase was prepared by the filtration of individual components⁴, mixing, and deaerating prior to use. All other solvents and reagents were analytical reagent quality or better. All extraction tubes, vials, and stage-2 Erlenmeyer flasks used in the analytical studies were silylated using 2% trimethylchlorosilane⁵ in toluene and were rinsed prior to use.

Analytical Methods—Analytical TLC was performed with silica gel plates⁶ or aluminum oxide plates⁷ in the following TLC systems: (a) silica gel developed in chloroform–methanol–acetone–28% ammonium hydroxide (63:7:27:2), (b) silica gel developed in chloroform–methanol–glacial acetic acid (13:6:1), (c) aluminum oxide developed in acetone, and (d) aluminum oxide developed in toluene–morpholine (18:2). Under these conditions, the following *R_f* values were observed: I (a) 0.81, (b) 0.91, (c) 0.95, and (d) 0.63; II (a) 0.54, (b) 0.44, (c) 0.52, and (d) 0.20; and III (a)

¹ Personal communications, E. C. Kornfeld, Eli Lilly Research Laboratories, 1978.

² Eli Lilly Co., Indianapolis, Ind.

³ OmniSolv, M.C.B. Reagents, Cincinnati, Ohio.

⁴ G/F grade glass fiber filter, Whatman, Clifton, N.J.

⁵ Aldrich, Milwaukee, Wis.

⁶ Polygram Sil G/UV 254, 0.25 mm, Brinkmann, Westbury, N.Y.

⁷ Polygram, Alox N/UV 254, 0.20 mm, Brinkmann, Westbury, N.Y.

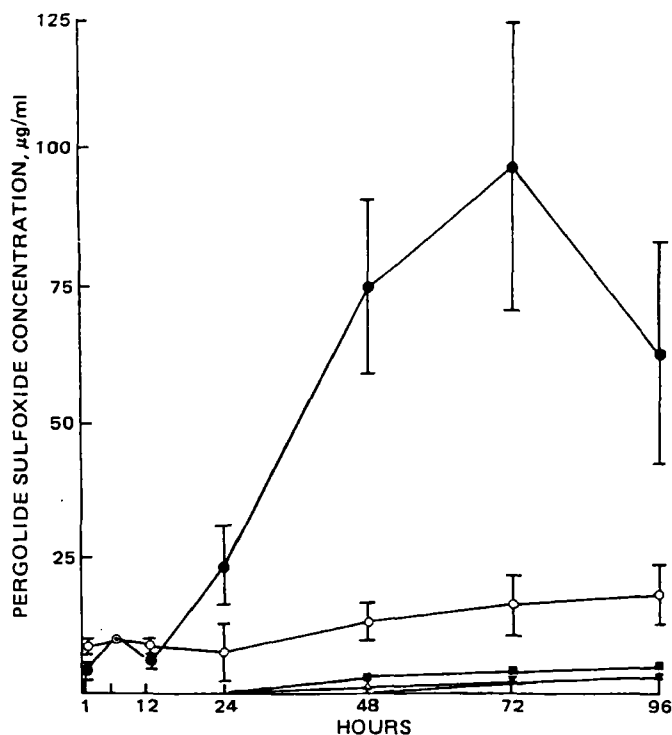


Figure 1—Enzymatic versus spontaneous air-oxidation of pergolide measured by HPLC in live *Helminthosporium* species NRRL 4671 cultures (●) and in controls consisting of autoclaved *Helminthosporium* species culture (○) and phosphate buffers at pH 4 (■), pH 7 (△), and pH 11 (▼). Determinations were made in triplicate; bars represent the SD of each mean value.

0.70, (b) 0.71, (c) 0.92, and (d) 0.39. Plates were visualized under UV light (254 nm) and sprayed with either Van Urks-Salkowski (modified) reagent for indoles (22), or Gibb's (23) and Pauly's (20) reagents in anticipation of hydroxylated metabolites (24). Screening experiments were monitored with TLC system *a*, and co-chromatography of the metabolites II and III with the standard materials was performed using all four TLC systems.

All analytical HPLC analyses were conducted as described previously (25) using an octadecyl reverse-phase 10- μ m 3.9 \times 300-mm column⁸. The mobile phase consisted of acetonitrile-0.01 M ammonium carbonate buffer pH 8.4 (3.75:2); the flow rate was 2 ml/min. Under these conditions, retention times were as follows: I, 6.74 min, II, 3.22 min, III, 2.31 min, and lergotril (internal standard), 2.42 min. Preparative HPLC was run using an octadecyl reverse-phase, 10- μ m, 8 \times 500-mm column⁹. The mobile phase was the same as above but used at 3 ml/min. Under these conditions, retention times were as follows: II, 11.98 min and III, 9.49 min.

Mass spectra¹⁰ (MS) gave the following results: I, *m/z* (% relative abundance), 314 (100), 299 (20), 285 (65), 267 (30); II (synthetic material and metabolite), *m/z* (% relative abundance), 330 (25), 315 (15), 313 (100), 301 (10), 267 (35); and III (synthetic material only), *m/z* (% relative abundance), 346 (100), 330 (3), 317 (89), 267 (22). ¹H-NMR spectra were recorded on a 100-MHz¹¹, or a 200-MHz spectrometer¹² using tetramethylsilane as the internal standard: I, δ (ppm) (deuteriochloroform), 2.16 [3, s, -SCH₃], 6.80-7.22 [4, aromatic protons], 7.95 [1, broad s, NH]; II (synthetic material and metabolite), δ (ppm) (deuteriochloroform), 2.64 and 2.63 [3, 2s, -SOCH₃], 6.80-7.22 [4, aromatic protons], 8.16 [1, broad s, NH]; and III (synthetic), δ (ppm) (deuteriochloroform), 3.00 [3, s, -SO₂CH₃], 6.8-7.2 [4, aromatic protons], 7.91 [1, broad s, NH]. Melting points for I (212.7-216.8°) and II (174.0-177.0°) were determined with a Fisher digital melting point analyzer¹³ at a rate of 2°/min and are uncorrected.

General Fermentation Conditions—All microorganisms were

maintained on refrigerated (4°) slants of either Sabouraud-maltose agar¹⁴, mycophyl agar¹⁵, or ATCC medium 5 sporulation agar¹⁴ and were transferred every 6 months to maintain viability. Incubations were performed in a two-stage fermentation procedure with soybean-dextrose medium (26). Appropriate substrates (I or II) were added to 24-hr stage-2 flasks. Portions (2 ml) were removed at 1, 2, 3, 6, and 10 days and extracted as described previously (26).

Preparative-Scale Production of Pergolide Sulfoxide (II) with *Helminthosporium* Species NRRL 4671—Six milliliters of stage-1 growth was used to inoculate each of six 250-ml stage-2 Erlenmeyer flasks containing 50 ml of soybean-dextrose medium (300 ml total), which were incubated at 27°, 250 rpm, for 24 hr. A total of 75 mg of pergolide mesylate was dissolved in 6 ml of sterile water and aseptically distributed among the flasks (0.25 mg/ml). After 5 days, the cultures were combined and homogenized, adjusted to pH 8.5 with an equal volume of 0.1 M sodium carbonate-sodium bicarbonate buffer (pH 8.5), and exhaustively extracted with ethyl acetate in a separatory funnel. The ethyl acetate extracts were combined, concentrated *in vacuo*, and partitioned against 0.05 N HCl. The acidic, aqueous extract was adjusted to pH 9 with 1 N NaOH and partitioned against ethyl acetate. The final ethyl acetate extracts were combined, concentrated *in vacuo* (520 mg), and chromatographed using preparative-scale TLC plates¹⁶. After development in TLC system *a* (*Analytical Methods*), the band representing II was scraped from the plate and eluted in a glass column with ethanol to remove the metabolite (46.2 mg). Compound II was recrystallized from ethanol (16.7 mg).

Triphenylphosphine Reduction of Pergolide Sulfoxide (II)—Following the procedure of Castrillon and Szmant (27), 1 mg of II was dissolved in 5.0 ml of carbon tetrachloride in a 1-ml conically tipped and capped vial¹⁷. Two equivalents of triphenylphosphine (1.66 mg) were added to the vial and the mixture was heated (90°) for 3 hr in a metal heating mantle¹⁸. After being concentrated under a nitrogen stream, the reaction mixture was analyzed by TLC and HPLC.

Peracid Oxidation of Pergolide (I) to Pergolide Sulfoxide (II)—Following the procedure of Curci and Modena (28), 5 mg of I was dissolved in 10 ml of 0.01 N HCl-ethanol (50:50); 2.7 mg of *m*-chloroperbenzoic acid (1 equivalent) was added and the reaction was allowed to proceed at room temperature. After a period of 4 hr, the ethanol was removed under a nitrogen stream, the mixture was adjusted to pH 9 with 1 N NaOH, and then extracted with ethyl acetate. The ethyl acetate extract was concentrated *in vacuo* to yield an oil which was analyzed by TLC and HPLC.

Peracid Oxidation of Pergolide (I) to Pergolide Sulfone (III)—Compound I (50 mg) and *m*-chloroperbenzoic acid (27 mg) were dissolved in 25 ml of 0.01 N HCl-methanol (50:50). After 24 hr, additional peracid (29.7 mg, 1 equivalent plus 10%) was added to the flask, and the reaction was allowed to proceed at 27° for 4 days. The products were extracted as described above and concentrated under a nitrogen stream. The resulting syrup was dissolved in 2 ml of mobile phase, and III was isolated by preparative HPLC, concentrated *in vacuo*, and purified by preparative-scale TLC (8.1 mg, oil) as described above for II.

Determination of the Enzymatic versus Spontaneous Air-Oxidation of Pergolide (I) by *Helminthosporium* species NRRL 4671—A 2-ml portion of a stage-1 culture of *Helminthosporium* species NRRL 4671 was used to inoculate each of seven stage-2 cultures which were generated in silylated 125-ml culture flasks¹⁹ containing 22 ml of medium. After incubating at 27° for 24 hr using a gyrorotary shaker²⁰ at 250 rpm, three of the cultures were autoclaved. Compound I (6.25 mg) in 1 ml of sterile water-ethanol (95:5) was added to each of the autoclaved and three of the live cultures. No substrate was added to the seventh flask to serve as a control for HPLC analyses. Triplicate control incubations were also conducted for I in 25 ml of autoclaved 0.1 M potassium phosphate buffer at pH 3, pH 7, and pH 11. A stock solution of the internal standard, lergotril (IV) (1 mg/ml of methanol), was prepared; a 100- μ l portion was placed in all extraction tubes, and the solvent was removed under a nitrogen stream. Two-milliliter portions of incubation samples were removed at 1, 6, 12, 24, 48, 72, and 96 hr; these were extracted and analyzed by HPLC as described previously (25). Results were expressed as amount of II produced (Fig. 1).

⁸ μ -Bondapak C-18, Waters Associates, Milford, Mass.

⁹ Lichrosorb RP-18, Whatman, Clifton, N.J.

¹⁰ Dupont Model 21491, Dupont Instruments Products Division, Wilmington, Del.

¹¹ Varian HA-100, Varian Associates, Palo Alto, Calif.

¹² Nicolet Model NT-200 Wide Bore, Nicolet Magnetics, Mountain View, Calif.

¹³ Fisher Scientific, Pittsburgh, Pa.

¹⁴ Difco Laboratories, Detroit, Mich.

¹⁵ B.B.L. Microbiological Systems, Cockeysville, Md.

¹⁶ Silica gel G, 1.0 mm, Analtech, Newark, N.J.

¹⁷ Reacti-Vial, Pierce Chemical Co., Rockford, Ill.

¹⁸ Reacti-Therm, Pierce Chemical Co., Rockford, Ill.

¹⁹ Bellco Delong culture flasks, Bellco Glass, Inc., Vineland, N.J.

²⁰ NBS Model G25-R Environmental Shaker, New Brunswick Scientific, Edison, N.J.

Table I—Microorganisms Examined for Their Ability to Effect the Biotransformation of Pergolide.

Microorganism	Source and Reference Number ^a
<i>Aspergillus alliaceus</i> ^b	UI 315
<i>A. foetidus</i>	NRRL 337
<i>A. niger</i>	UI-X-172
<i>A. niger</i> ^b	ATCC 16888
<i>A. niger</i>	ATCC 10581
<i>A. niger</i>	ATCC 10548
<i>A. oryzae</i>	NRRL 447
<i>Beauveria bassiana</i>	ATCC 13144
<i>B. sulfurescens</i>	ATCC 7159
<i>Calonectria decora</i> ^b	ATCC 14767
<i>Cryptococcus mascerans</i>	Ziffer
<i>Cunninghamella bainieri</i>	UI 3065
<i>C. bertholletiae</i>	NRRL 3644
<i>C. bertholletiae</i>	ATCC 11064
<i>C. blakesleeana</i>	NRRL 1369
<i>C. blakesleeana</i>	ATCC 8688a
<i>C. blakesleeana</i>	UI Sih-2138
<i>C. echinulata</i> ^b	ATCC 9244
<i>C. echinulata</i> ^b	UI Sih-1387
<i>C. echinulata</i> ^b	UI 3655
<i>C. echinulata</i> ^b	UI Sih-1386
<i>C. echinulata</i> ^b	ATCC 11585a
<i>C. echinulata</i> ^b	ATCC 11585b
<i>C. elegans</i>	ATCC 9245
<i>C. elegans</i>	UI Sih-1393
<i>Curvularia lunata</i>	ATCC 13633
<i>Gliocladium deliquescens</i>	UI 1086
<i>Helicostylum piriforme</i>	UI-2-QM 6945
<i>H. piriforme</i> (+)	UI-QM 6945
<i>H. piriforme</i> (-)	UI-QM 6944
<i>Helminthosporium</i> species	NRRL 4671
<i>Microsporium gypseum</i>	ATCC 11395
<i>Mortierella isabellina</i>	Abushanab
<i>Mucor mucedo</i>	UI 4605
<i>Saccharomyces cerevisiae</i>	NRRL Y-2034
<i>Schizosaccharomyces pombe</i>	ATCC 2476
<i>S. pombe</i>	ATCC 20130
<i>Sepedonium chrysospermum</i>	ATCC 13378
<i>Sporobolomyces pararoseus</i>	ATCC 11386
<i>Streptomyces albogriseolus</i>	NRRL B-1305
<i>S. aureofaciens</i>	ATCC 13304
<i>S. flocculus</i>	ATCC 25453
<i>S. griseus</i>	ATCC 10137
<i>S. griseus</i>	NRRL B-599
<i>S. griseus</i>	NRRL 3242
<i>S. griseus</i>	UI 1158W
<i>S. lavendulae</i>	NRRL B-2036
<i>S. lavendulae</i>	UI Sih L-105
<i>S. lincolnensis</i>	ATCC 25466
<i>S. lincolnensis</i>	NRRL 2936
<i>S. paucisporogenes</i>	ATCC 12596
<i>S. platensis</i>	NRRL 2364
<i>S. punipaulis</i>	NRRL 3529
<i>S. purpurascens</i>	ATCC 21326
<i>S. rimosus</i>	ATCC 23955
<i>S. rimosus</i>	NRRL 2234
<i>S. rutgersensis</i>	NRRL B-1256
<i>S. scabies</i>	UI Sih-1627
<i>Streptomyces</i> species	UI MR-127

^a Key: (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; (Abushanab) received from Dr. E. Abushanab, University of Rhode Island, Kingston, R.I.; (Ziffer) received from Dr. H. Ziffer, National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Md. ^b These microorganisms formed both pergolide sulfoxide (II) and pergolide sulfone (III).

Determination of the Enzymatic versus Spontaneous Air-Oxidation of Pergolide Sulfoxide (II) to Pergolide Sulfone (III) by *Aspergillus alliaceus* UI 315—A 2-ml portion of the stage-1 culture of *Aspergillus alliaceus* UI 315 was used to inoculate each of three stage-2 culture flasks containing 25 ml of medium. After incubating at 27° for 24 hr using a gyratory shaker at 250 rpm, one of the cultures was autoclaved. Compound II (6.25 mg) in 1 ml of sterile water-ethanol (95:5) was added to the autoclaved and live cultures and to sterile media used as a control. A fourth culture (without added substrate) served as a control in the TLC analyses. Two-milliliter portions of incubation samples were removed at 1, 2, 3, 6, and 10 days, extracted, and analyzed by TLC system *a*.

RESULTS AND DISCUSSION

The metabolism of many xenobiotics by microorganisms parallels their metabolism in mammalian systems. The observation that xenobiotics are modified chemically in a highly selective way by microorganisms (29) has formed the basis for studies of microbial models of mammalian metabolism (20, 21). A desire to produce sufficient quantities of metabolites of the ergoline pergolide (I) for biological evaluation prompted us to examine 58 microorganisms for their ability to effect the biotransformation of I (Table I). These microorganisms were chosen based on their reported ability to catalyze oxidations of sulfur or affect indole hydroxylations (30-34). A majority of the microorganisms investigated formed pergolide sulfoxide (II), a known mammalian metabolite; several (*A. alliaceus* UI 315, *Aspergillus niger* ATCC 16888, *Calonectria decora* ATCC 14767, and all *Cunninghamella echinulata* strains) formed another known metabolite, pergolide sulfone (III), in addition to II. Additional studies were performed with *Helminthosporium* species NRRL 4671, since it yielded significant amounts of II from I. *A. alliaceus* UI 315 was employed to study the formation of III.

A preparative-scale incubation of I with the *Helminthosporium* species allowed the identification of the major metabolite, II, based on the MS and ¹H-NMR spectral data. The melting point of the metabolite was identical to authentic sulfoxide and did not change during mixed melting point determinations. The products of the triphenylphosphine reduction of II and the peracid oxidation of I were cochromatographed with I and II, respectively, in TLC system *a* and by HPLC. Cochromatography of the metabolite with authentic sulfoxide in four TLC systems provided additional evidence of its identity. Characterization of III was based on cochromatography of this metabolite obtained from *A. alliaceus* microbial extracts with synthetic III in all four TLC systems and by its HPLC retention time.

Verification that III was formed enzymatically was accomplished through an analytical experiment in which II was incubated with *A. alliaceus* cultures. The formation of III and I in active cultures, as determined by TLC, indicated that III formed from I via the sulfoxide. The fact that sulfide oxidation was reversible and that III did not form in control incubations supports enzyme-mediated reactions.

The problem of the air-oxidation of I to II necessitated a study of the enzymatic versus spontaneous air-oxidation of I. HPLC was chosen as the method of analysis based on its demonstrated utility in the rapid analysis of microbial extracts (35). This experiment (Fig. 1) illustrated the large difference between the enzymatic (40%) oxidation of I by *Helminthosporium* species and the maximum spontaneous air-oxidation (6%) of the drug. The substrate, I, was converted maximally after 48 hr, and there were no significant differences (*F* test) in product formation during subsequent time periods.

The utility of the microbial production of pergolide metabolites for biological evaluation was varied. The apparently low yield in the transformation of I to III by *A. alliaceus* makes the chemical preparation of this compound a more viable procedure. There are many examples of the stereoselective formation of sulfoxides by microorganisms (36). However, only minor (if any) stereoselectivity in the formation of one pergolide sulfoxide diastereomer by *Helminthosporium* species was suggested from ¹H-NMR data (diastereomeric thiomethyl signals). Other organisms producing the sulfoxide might be examined for a higher degree of stereoselectivity.

In summary, an investigation of 58 microorganisms for their ability to effect the biotransformation of the semisynthetic ergoline alkaloid, pergolide, indicated that a majority produced the known mammalian metabolite pergolide sulfoxide (II), and that several microorganisms formed a second mammalian metabolite, pergolide sulfone (III), in addition to II. These results further support the concept of microbial models of mammalian metabolism.

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Stereospecific Radioimmunoassays for *l*-Ephedrine and *d*-Ephedrine in Human Plasma

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Abstract □ Haptens were prepared by the reaction of *d*-ephedrine or *l*-ephedrine with methyl acrylate and subsequent alkaline hydrolysis of the methyl ester groups. The haptens were coupled to bovine serum albumin by a mixed anhydride method, and the resulting drug-protein conjugates were used to immunize rabbits. Antisera raised to these conjugates were highly stereospecific. Neither antiserum cross-reacted with the optical antipode of its substrate nor with racemic pseudoephedrine. Separate radioimmunoassays (RIAs), developed for *d*-ephedrine and *l*-ephedrine, were used to measure the concentrations of the enantiomers of ephedrine in the blood of two volunteers dosed with racemic ephedrine. The RIAs were validated by comparing the sum of the concentrations of the enantiomers, determined by RIA, with total ephedrine concentrations determined by a nonstereoselective GLC-ECD method.

Keyphrases □ Ephedrine—stereospecific radioimmunoassay using (*d*) and (*l*) antisera □ Radioimmunoassay—for *d*- and *l*-ephedrine, stereospecific □ Antisera—for *d*- and *l*-ephedrine, cross-reactivity, use in stereospecific radioimmunoassays

2-Methylamino-1-phenylpropanol has two chiral centers which give rise to four optical isomers. Racemic ephedrine is a mixture of the erythro pair of diastereomers (*l*), (1*R*,2*S*) and (*d*), (1*S*,2*R*), while the threo pair of diastereomers, (*l*), (1*S*,2*S*) and (*d*), (1*R*,2*R*) is called pseudoephedrine (1, 2). The four optical isomers differ from each other in their pharmacological activities (3–7) and rates of metabolism (8–13).

Preparations containing *l*- or *dl*-ephedrine are used for a wide variety of therapeutic applications, such as the treatment of nasal congestion in colds and allergic rhinitis, the treatment of orthostatic hypotension, as mydriatics, and as prophylactics against asthma attacks, urinary incontinence, and motion sickness (14). Of the published analytical methods for ephedrine (15–21), only GLC-ECD (22, 23) and GLC-MS (24) are applicable to the measurement of therapeutic concentrations in plasma; none of the methods distinguish between the enantiomers of ephedrine. Recently however, Findlay *et al.* (25) developed a stereospecific radioimmunoassay (RIA) for *d*-pseudoephedrine. This paper describes the development of separate RIAs for *l*-ephedrine and *d*-ephedrine and their validation by comparison with a GLC-ECD method.

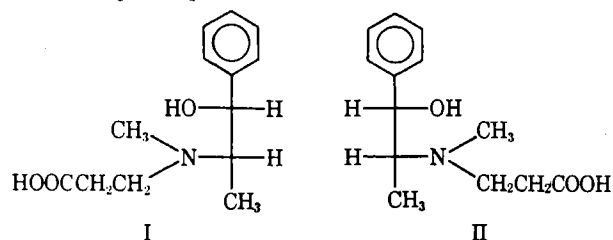


Figure 1—Haptens for *l*-ephedrine (I) and *d*-ephedrine (II).