Journal of Chromatography, 219 (1981) 317–320
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

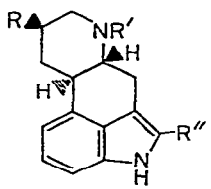
CHROM. 14,240

Note

High-performance liquid chromatographic determination of pergolide and its metabolite, pergolide sulfoxide, in microbial extracts

KATHLEEN M. KERR*, ROBERT V. SMITH and PATRICK J. DAVIS
Drug Dynamics Institute, and Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Texas at Austin, Austin, TX 78712 (U.S.A.)
(Received July 28th, 1981)

The pharmacological effects of ergot alkaloids are well documented, and the motive for recent research with ergoline derivatives has been to design drugs for the treatment of prolactin-dependent disorders, such as galactorrhea and amenorrhea, prolactin-dependent breast tumors, and Parkinson's disease[1–3]. During a study undertaken to define the structural requirements necessary for prolactin inhibition[4], the drug pergolide (I) (see Fig. 1) was synthesized and was later found to be one of the most potent *in vitro* and *in vivo* dopamine agonists and inhibitors of prolactin secretion[4–6]. Clinical studies indicated that pergolide may be efficacious in the treatment of prolactin-dependent disorders[7].



I. R = CH_2SCH_3, R′ = $CH_2CH_2CH_3$, R″ = H

II. R = $CH_2\overset{O}{\uparrow}SCH_3$, R′ = $CH_2CH_2CH_3$, R″ = H

III. R = $CH_2C{\equiv}N$, R′ = CH_3, R″ = Cl

Fig. 1. Structures of pergolide (I), pergolide sulfoxide (II) and lergotrile (III).

We are currently examining the use of microorganisms to prepare metabolites of pergolide for biological evaluation and for comparison to metabolites found in mammalian species. Studies involving the microbial transformation of pergolide sulfoxide (II) by a *Helminthosporium* species (NRRL 4671) has necessitated the development of a new analytical procedure for pergolide and its metabolite; thus, high-performance liquid chromatography (HPLC) was chosen for its demonstrated utility in the rapid analysis of microbial extracts[8]. The developed method may be useful in other pharmaceutical, pharmacokinetic, and/or clinical studies with ergoline derivatives.

0021-9673/81/0000–0000/$02.50

EXPERIMENTAL

Materials

Pergolide (I), pergolide sulfoxide (II) and lergotrile (III) were provided by Eli Lilly, Indianapolis, IN, U.S.A. Ergoline spectra (mass spectra, proton magnetic resonance spectra) were consistent with anticipated results, as were physical data (m.p.). The compounds were found to be homogeneous by thin-layer chromatography and HPLC.

Water for use in HPLC was deionized and double-distilled in glass; acetonitrile was HPLC-grade (OmniSolv; MCB Reagents, Cincinnati, OH, U.S.A.). The mobile phase was prepared by the filtration of individual solvents through glass fiber filter pads (GF/F grade; Whatman, Clifton, NJ, U.S.A.), mixing and degassing prior to use. All other solvents and reagents were analytical reagent quality.

All glassware used in extractions was silylated using 2% trimethylsilylchloride (TMSCl) (Aldrich, Milwaukee, WI, U.S.A.) in toluene, rinsed thoroughly and dried prior to use.

Chromatographic procedure

A Beckman Model 110A pump, an Altex Model 210 injector with a 50-μl sample loop (Beckman, Silver Springs, MD, U.S.A.) and a Tracor Model 970 variable-wavelength detector (Tracor, Austin, TX, U.S.A.) were employed for all HPLC analyses. An Altex Model C-R1A integrator (Beckman) at an input sensitivity of 1 mV/min was used for peak area measurements and chromatographic recording. A μBondapak C_{18} column, 300 × 3.9 mm I.D. (Waters Assoc., Milford, MA, U.S.A.), was used. 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 (1000 p.s.i.). Under these conditions, retention times were as follows: pergolide, 6.74 min; pergolide sulfoxide, 3.22 min; and lergotrile, 2.42 min.

Helminthosporium cultivation

The *Helminthosporium* species (NRRL 4671) was maintained on mycophyll agar slants (BBL, Cockeysville, MD, U.S.A.) which were refrigerated at 4°C. Incubations were performed in a two-stage fermentation procedure as described earlier[9]. Cultures were harvested, combined and fully homogenized (Polytron; Brinkmann, Westbury, NY, U.S.A.) before HPLC analysis.

Extraction and analysis

Spiked samples were prepared using a single extraction step. Stock solutions at levels of 1 mg/ml in methanol were prepared for pergolide, pergolide sulfoxide and lergotrile. Portions of 500, 250, 150, 100, 50 and 20 μl of pergolide and pergolide sulfoxide solutions and 100 μl of the internal standard solution of lergotrile, were placed in 125 × 16 mm silylated glass extraction tubes and the solvent was removed under a nitrogen stream. A total of 2 ml of the *Helminthosporium* culture homogenate was added to the tube and the mixture was alkalinized with 2 ml of 0.1 *M* sodium carbonate–sodium bicarbonate buffer (pH 8.5) and extracted with 4 ml of isoamyl alcohol. Samples were agitated for 30 min (30 oscillations per min) on a Lab-Tek aliquot mixer, and centrifuged (1230 *g*) for 10 min before 2 ml of the isoamyl alcohol

layer were removed and dried under a nitrogen stream; samples containing 250 μg/ml of I and II were reconstituted in 500 μl of mobile phase; the remainder of all samples was dissolved in 250-μl portions of mobile phase, filtered and subjected to HPLC analysis.

Samples at levels of 250, 125, 75, 50, 25 and 10 μg/ml of pergolide and pergolide sulfoxide were prepared without extraction for comparison of peak areas with extracted samples for the calculation of absolute recovery values. The absolute recovery of lergotrile was determined at a level of 100 μg/ml only. Resultant peak areas were used to prepare standard curves where peak area ratios (standard/lergotrile) were plotted *vs.* μg of standard compound per ml of culture.

RESULTS AND DISCUSSION

Fig. 2 illustrates the HPLC separation system developed for pergolide (I), pergolide sulfoxide (II) and the internal standard, lergotrile (III). A series of indole compounds (*e.g.*, tryptamine) were examined as possible internal standards, and lergotrile was chosen based on its structural similarity, resolution from all peaks in the chromatogram and absolute recovery from isoamyl alcohol (91.1 ± 2.9%, $n = 6$). The wavelength chosen for detection (290 nm) was optimal for all three compounds.

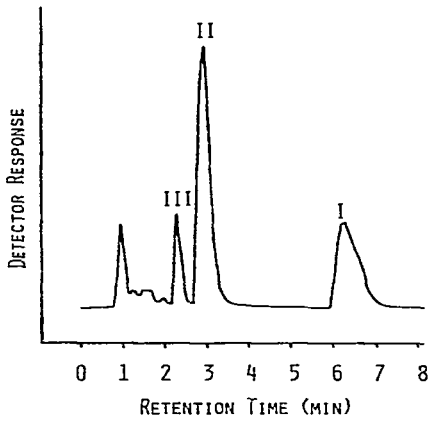


Fig. 2. Chromatographic separation of pergolide (I), pergolide sulfoxide (II) and lergotrile (III) from a culture extract of *Helminthosporium*. Chromatographic conditions are described in the Experimental section.

The final composition of the developed mobile phase resulted from systematic variation of the components in the mixture until adequate resolution was obtained while retaining a short analysis time (8 min). Alkaline conditions (pH 8.4) were chosen to allow for development of totally unionized species and did not appear to effect the integrity of the HPLC column over a period of nine months.

A simple extraction procedure was developed which allows for the HPLC analysis of a *Helminthosporium* culture after a single extraction step. Culture homogenates were spiked with levels of standards of pergolide and pergolide sulfoxide based on maximum substrate levels and theoretically maximal product formation (250 μg/ml). Isoamyl alcohol was chosen for extraction based on absolute recovery experi-

ments with an array of organic solvents (chloroform, dichloromethane, diethyl ether, ethyl acetate) and a lack of interference from co-extracted media components. Good absolute recoveries of pergolide (71.2 ± 7.6%, $n = 6$) and pergolide sulfoxide (91.5 ± 5.2%, $n = 6$) were obtained with this solvent. Resulting standard curves for pergolide and pergolide sulfoxide produced satisfactory results; a typical pergolide standard curve yielded a slope of 0.0128 (y-intercept = 0.0685, $r = 0.9954$) and the pergolide sulfoxide standard curve typically yielded a slope of 0.0244 (y-intercept = −0.0096, $r = 0.9997$). This demonstrates the utility of this analysis over a concentration range anticipated for both compounds in microbial transformation experiments.

In summary, a method has been developed for the simple extraction and rapid analysis of pergolide and its metabolite, pergolide sulfoxide, in *Helminthosporium* cultures. This method has been applied to growing cultures of a *Helminthosporium* species to determine the amount of enzymatic *vs.* spontaneous air-oxidation of pergolide (in preparation). This method may have application in the analytical determination of additional ergolines and their metabolites, and the preparative-scale separation of these compounds.

ACKNOWLEDGEMENT

This research was supported in part by the Eli Lilly Research Foundation.

REFERENCES

1 D. B. Calne, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 37 (1978) 2207.
2 H. G. Floss, J. M. Cassady and J. E. Robbers, *J. Pharm. Sci.*, 62 (1973) 699.
3 L. Lemberger, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 37 (1978) 2176.
4 R. W. Fuller, J. A. Clemens, E. C. Kornfeld, H. D. Snoddy, E. B. Smalstig and N. J. Bach, *Life Sci.*, 24 (1979) 375.
5 G. Delitala, J. Yeo, A. Grossman, N. R. Hathaway and G. M. Besser, *J. Endocrinol.*, 87 (1980) 95.
6 J. G. Cannon, B. J. Demopoulos, J. P. Long, J. R. Flynn and F. M. Sharabi, *J. Med. Chem.*, 24 (1981) 238.
7 J. T. Callaghan, R. E. Cleary, R. Crabtree and L. Lemberger, *Life Sci.*, 28 (1981) 95.
8 P. J. Davis, *J. Chromatogr.*, 193 (1980) 170.
9 P. J. Davis, J. C. Glade, A. M. Clark and R. V. Smith, *Appl. Environ. Microbiol.*, 38 (1979) 891.