Identification and Purity Test for Ergonovine and Methylergonovine

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A method is described for the separation and identification of those "water-soluble" ergot alkaloids of pharmaceutical interest; namely, ergonovine, ergonovinine, methylergonovine, and methylergonovinine.

THE IDENTIFICATION tests in the official compendia (1, 2) fail to distinguish between ergonovine (the principal oxytocic agent of ergot), methylergonovine (a synthetic homolog), and the dextrorotatory, physiologically inactive isomers, ergonovinine and methylergonovinine. This study was undertaken for the purpose of developing a suitable test for the identification of ergonovine and methylergonovine in pharmaceuticals, and for the detection of possible contaminants and breakdown products. In searching for such a test, several of the paper chromatographic procedures reported in the literature were investigated. With each of the partitioning procedures described by Foster, *et al.* (3), Macek (4), Voigt and Weiss (5), and Alexander and Banes (6) we could effectively separate the dextrorotatory alkaloids from their diastereoisomers. Attempts to separate ergonovine and methylergonovine from each other by these and similar procedures were unsuccessful. The reverse-phase paper chromatographic procedure described by Stoll and Rüegger (7) included an overnight equilibration of the spotted paper in the vapors of the mobile phase. It was our experience that, with this procedure, each of the isomers would partially convert to its diastereoisomer. Pöhm and Fuchs (8) and Horak and Kudrnac (9) described methods incorporating benzene into the mobile solvent. In the hands of the author, these ergot alkaloids decomposed in the presence of benzene.

Berg (10) described a method for the separation of water-soluble ergot alkaloids as well as the products of hydrolysis, ergine, and lysergic acid. Circular wick paper chromatographic technique was used. Papers were impregnated with pH 6.4 buffer and chromatograms developed with water-saturated methylene chloride. The method described below is an adaptation of Berg's system incorporating the simpler ascending paper chromatographic techniques described by Mitchell (11) and the "moist-paper" technique described by Levine and Fischbach (12).

MATERIALS AND METHOD

Mobile Solvent.—Mix 50 ml. of ethylene chloride, 50 ml. of *n*-butyl acetate, 7 ml. of pyridine, and 2 ml. of water in a separator. Allow to settle, draw off the bottom layer through a glass wool filter, and discard the top layer.

Standard Solutions.—Dissolve 10 mg. of methylergonovine maleate N.F. XI, or ergonovine maleate U.S.P. XVI in 2.0 ml. of alcohol. This is *standard solution A*. Dilute 1.0 ml. of standard solution A to 25 ml. with alcohol to obtain *standard solution B*.

Sample Solution.—Dissolve ergonovine maleate or methylergonovine maleate powder in a suitable quantity of alcohol to obtain a solution strength of 5 mcg. per μl. To analyze an injection solution, render a suitable portion alkaline with sodium bicarbonate, extract with chloroform, evaporate the extract to dryness, and dissolve the residue in a quantity of alcohol sufficient to obtain a solution strength equivalent to 5 mcg. ergonovine maleate or methylergonovine maleate per μl. To analyze tablets, prepare chloroform extracts as described under *Identification* in the N.F. monograph for methylergonovine maleate tablets (2). Dissolve the dried residues in a quantity of alcohol sufficient to obtain a solution strength equivalent to 5 mcg. per μl.

Procedure.—Arrange a chromatographic tank suitable for ascending paper chromatography. Add mobile solvent, seal the tank, and equilibrate for 1 hour. Pour about 10 ml. aqueous buffer solution pH 4.6 [such as U.S.P. phthalate buffer (13)] over premarked paper. After draining for 30 seconds, place the paper between sheets of paper toweling. Blot the surface with the toweling for some seconds to remove as much moisture as will be absorbed, and repeat using fresh towels. Spot 0.010 ml. of each of the standard solutions and volumes of sample solutions equivalent to 50 mcg. ergonovine maleate or methylergonovine maleate. Rewet the spots with water from a capillary tube. In addition, spot methyl red U.S.P. XVI, T.S., at the edge of the paper to aid in following the solvent front. The paper should feel definitely moist and limp, but not so wet as to transfer water to the fingers. Place the paper in the chromatographic tank and reseal.

When the solvent front is within 2 cm. of the top of the paper (about 1 hour), remove it and dry in a hood. Examination under ultraviolet light should show one primary spot with an R_f and an intensity corresponding to that of the respective standard A spot. Any other spot occurring on the sample chromatogram represents impurities. The standard B (2 mcg.) spot is used to estimate roughly the quantity of any such impurities.

The lysergic acid moiety of these ergot alkaloids will react with strongly acidic *p*-dimethylaminobenzaldehyde to give a blue color. By spraying the developed chromatogram with an alcoholic, acidic solution of this aldehyde, as described by Hellburg (14), and photographing the dried chromatogram, permanent records may be obtained.

RESULTS AND DISCUSSION

Typical R_f values obtained are 0.19 for ergonovine, 0.35 for methylergonovine, 0.44 for ergonovinine, and 0.60 for methylergonovinine. Any water-insoluble ergot alkaloids present as contaminants will be indicated by spots located at the solvent front. Ergonovinine and methylergonovinine used in the study were prepared by the method described by Kleiderer (15) whereby 5 mg. of the levorotatory isomer is dissolved in 1 ml. of methyl alcohol, two drops of concentrated ammonia added, and the solution kept at room temperature overnight.

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About half of the alkaloids are converted to the dextrorotatory form by this procedure.

When attempting to adapt Berg's system to a simpler apparatus, ergonovine and methylergonovine were well separated; but spots were very diffuse and the developing solvent failed to ascend the paper evenly. More satisfactory chromatograms were obtained by diluting the developing solvents with *n*-butyl acetate. Less volatile chlorinated solvents would not separate ergonovine and methylergonovine. Of the volatile chlorinated solvents, only methylene chloride or ethylene chloride would separate the homologs and then only when they constituted half, or more, of the developing solvent.

By simultaneously increasing the acidity of the impregnating solution and the basicity of the mobile solvent as compared to conditions prescribed by Berg, less diffuse spots were obtained while maintaining about the same R_f values. In analyzing alkaloid samples by the procedure described above, unsatisfactory chromatograms were occasionally obtained where the alkaloids had migrated excessively or insufficiently during development. The mobility of the alkaloids could be increased by raising the pH of the mobile solvent or decreased by lowering the pH. The R_f values reported are typical of those obtained. When desired for special cases, greater than usual separation of ergonovine and methylergonovine, or greater separation of a pair of diastereoisomers, can be achieved by such a pH adjustment.

Several techniques were tried for conditioning the paper prior to development. Among these is the commonly used technique whereby papers are impregnated, dried, spotted, moistened by equilibration, and developed. Most satisfactory results were obtained by the "moist-paper" technique described above. Grossly diffuse spots are obtained if the papers are too wet when placed in the tank and immobility of the spots will result if the paper is too dry.

A methylergonovine maleate tablet mix and several injection solutions, of both ergonovine maleate and methylergonovine maleate, were prepared to simulate market products. These were then analyzed by the procedure described in this paper. The results indicated that there is no deterioration of alkaloid during analysis and that the excipients do not interfere. The procedure described in this paper was successfully applied to 14 commercial samples. Neither of the four samples of alkaloid salts, nor any of the seven tablet samples analyzed, contained more than trace quantities (about 1%) of the respective diastereoisomer. Only one of three injection solutions tested contained any dextrorotatory isomer and that, only after a year of storage under refrigeration. The chromatogram for another one of these injection solutions exhibited faint spots representing material not containing the lysergic acid moiety. These may have represented oxidized alkaloids.

REFERENCES

(1) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1960, p. 266.
(2) "The National Formulary," 11th ed., J. B. Lippincott Co., Philadelphia, Pa., 1960, p. 228.
(3) Foster, G. E., Macdonald, J., and Jones, T. S. G., *J. Pharm. and Pharmacol.*, **1**, 802(1949).
(4) Macek, K., *Pharmazie*, **9**, 752(1954).
(5) Voigt, R., and Weiss, F., *ibid.*, **13**, 212(1958).
(6) Alexander, T. G., and Banes, D., THIS JOURNAL, **50**, 201(1961).
(7) Stoll, A., and Rüegger, A., *Helv. Chim. Acta*, **37**, 1725(1954).
(8) Pöhm, M., and Fuchs, L., *Naturwissenschaften*, **41**, 63(1954).
(9) Horak, P., and Kudrnac, S., *Ceskoslov. Farm.*, **5**, 595(1956); through *Anal. Abstr.*, **4**, 4103(1957), and *Mfg. Chemist*, **29**, 160(1958).
(10) Berg, A. M., "Chromatographische Scheiding der Möderkoornalkaloïden en een Practische Toepassing Daarvan," Uitgeverij Excelsior 's-Gravenhage.
(11) Mitchell, L. C., *J. Assoc. Offic. Agr. Chemists*, **40**, 999(1957).
(12) Levine, J., and Fischbach, H., THIS JOURNAL, **44**, 543(1955).
(13) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1960, p. 929.
(14) Hellberg, H., *Acta Chem. Scand.*, **11**, 219(1957).
(15) Kleiderer, E. C., *J. Am. Chem. Soc.*, **57**, 2007(1935).

ERRATUM

In the paper titled *"In Vitro* Testing of Timed Release Tablets and Capsules" (1), the address of the manufacturer under Fig. 1 should read Ernest D. Menold, 5th and Powhatan Ave., Lester, Pa.

(1) Krueger, E. O., and Vliet, E. B., THIS JOURNAL **51**, 181(1962).

ERRATUM

In the paper titled "Study of the Boric Acid-Glycerin Complex II" (1), the formulas on page 238 should read

$$\frac{d \ln S}{dT} = \frac{\Delta H}{RT^2}$$

which when integrated becomes

$$\log S = \frac{-\Delta H}{2.303\,RT} + C$$

(1) Sciarra, J. J., and Monte Bovi, A. J., THIS JOURNAL, **51**, 238(1962).