Thin-Layer Chromatography of Ergot Alkaloids

By J. L. McLAUGHLIN, J. E. GOYAN, and A. G. PAUL

A technique for qualitative identification and quantitative determination of micro quantities of ergot alkaloids has been developed. Using glass plates spread with a layer of silica gel G and a solvent system composed of benzene:dimethylformamide (13:2) or of ethyl acetate:dimethylformamide:ethanol (13:1.9:0.1), one can separate ergonovine, ergotamine, ergosine, ergocristine, and a mixed zone composed of ergocornine and ergokryptine. The mixed zone is removed from the plate, the alkaloids eluted with 10 per cent methanol in chloroform, and filtered or centrifuged to remove the silica gel. The eluant is evaporated, and the mixture of the alkaloids is dissolved in a standard volume of methanol-chloroform. The respotting of this solution on glass plates spread with a layer of aluminum oxide G and chromatographed with a solvent system composed of chloroform:ether:water (3:1:1) results in the separation of ergocornine and ergokryptine. These solvent systems can be utilized to separate the isomers of the six alkaloids with equal facility. The technique has been extended to the resolution and quantitative estimation of the alkaloids.

THESE INVESTIGATIONS were undertaken to develop a rapid technique for the qualitative identification and quantitative estimation of 12 ergot alkaloids in micro amounts. The technique of thin-layer chromatography (TLC) was chosen because of its advantages over paper partition chromatography in requiring shorter time of equilibration and shorter time of development. In addition, 18 spots can be chromatographed on one plate, 8 × 8 in., and resolved components of mixtures are easily recovered from the developed chromatogram by elution.

A procedure reported by Teichert, *et al.* (1), combines some of the advantages of TLC with the versatility of paper partition chromatography using cellulose layers for the separation of the water insoluble ergot alkaloids. This procedure is useful for qualitative identification of ten of the 12 alkaloids. However, the R_f values of ergocorninine and ergocristinine, and of ergokryptine and ergocornine are almost identical. This prohibits use of this technique as a quantitative tool. Other reports using adsorption TLC show only group-wise separation of the alkaloids (1, 2).

The clavine types of ergot alkaloids, which are found in saprophytic cultures and in certain species of the fungus, have been successfully separated by TLC and paper chromatography (3–5).

EXPERIMENTAL

Apparatus.—The RSCo model A-200 chromatofilm assembly for TLC was used in these investigations.[1] Glass plates were 8 × 8 in.

Reagents.—Chemicals used in these investigations were reagent grade or equivalent, except for the following: *N,N*-dimethylformamide and *p*-dimethylaminobenzaldehyde were obtained from Eastman Organic Chemicals, and silica gel-G (SGG) and aluminum oxide-G (Al_2O_3G) were obtained from the Research Specialties Co.[1]

Preparation of Plates.—A slurry was prepared by mixing 25 Gm. of the adsorbent (SGG or Al_2O_3G) with 35 ml. of deionized water to a uniform consistency. An additional 15 ml. of deionized water was added to the slurry and the mixture stirred. The final slurry was poured into the spreader, and this in turn was rapidly drawn over the glass plates arranged on the aligning tray.[2] The plates were permitted to air-dry for approximately 5 minutes, placed in a storage rack, and activated for at least 30 minutes at 105°. Plates were stored at 105° until used.

Just prior to use, 7 mm. of adsorbent was scraped from the starting edge of each plate with a razor blade, using a plastic template as a guide.

Plates used for the determination of R_f values were scored with a sharp stylus in the direction of solvent development at 1-cm. intervals. This scoring of plates yields 18 individual strips of adsorbent and reduces the drift of zones due to irregularities in thickness of the adsorbent layer.

Standard Solutions.—The alkaloids[3] used in these investigations included ergonovine, ergometrinine, ergotamine, ergotaminine, ergosine, ergosinine, ergocristine, ergocristinine, ergokryptine, ergokryptinine, ergocornine, and ergocorninine. Solutions were prepared by dissolving 1.00 mg. of each alkaloid, weighed with a Cahn model M-10 electrobalance,[4] in 10% methanol in chloroform and diluting to 1.0 ml. in a volumetric flask. Mixtures of ergocornine and ergokryptine and of all 12 alkaloids were prepared in the same concentration as well. The solutions of alkaloids were protected from light and stored at refrigerator temperature. Solutions of alkaloids used for quantitative studies were prepared just prior to use.

Solvent Systems.—Of the 111 solvent systems tested, the following were most effective in separat-

Received June 3, 1963, from the College of Pharmacy, University of Michigan, Ann Arbor.
Accepted for publication July 26, 1963.
This investigation was supported in part by Public Health Service fellowship GF-14694 and in part by Grant RG-9491, Division of General Medical Sciences, U. S. Public Health Service, Bethesda, Md.
Presented to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.

[1] Research Specialties Co., Richmond, Calif.

[2] The fixed-thickness spreader employed delivers a layer of slurry approximately 250 μ thick.

[3] Ergotamine obtained from Mann Research Laboratories, New York, N. Y. All other alkaloids obtained from Sandoz Pharmaceuticals, Hanover, N. J.

[4] Cahn Instrument Co., Paramount, Calif.

ing the ergot alkaloids tested: ethylacetate:*N,N*-dimethylformamide:ethanol, 13:1.9:0.1 (*A*); benzene:*N,N*-dimethylformamide, 13:2(*B*); chloroform:ethyl ether:water, 87.5·12.5:25(*C*); and chloroform:ethyl ether:water, 3:1:1(*D*). The first two solvent systems were mixed at 21 ± 0.5° and used immediately. Solvent systems *C* and *D* were mixed, shaken vigorously, and allowed to equilibrate for 18 to 24 hours at 21 ± 0.5°. The organic phase was separated from the aqueous phase just prior to use.

Application of Test Solutions.—Solutions of the alkaloids were applied to the plates 2 cm. from the starting edge, using Kirk micropipets. For studies of qualitative separation of the alkaloids, 2 μl. of each solution was spotted in successive portions to assure a zone no greater than 5 mm. in diameter using a template as a guide. In the studies of quantitative separations of the alkaloids and in qualitative confirmation of ergokryptine and ergocornine, solutions were applied as a series of spots, using a 10-μl. Kirk micropipet, resulting in a band of the test compound. Again, the rate of application was controlled to assure a band width of no greater than 5 mm.

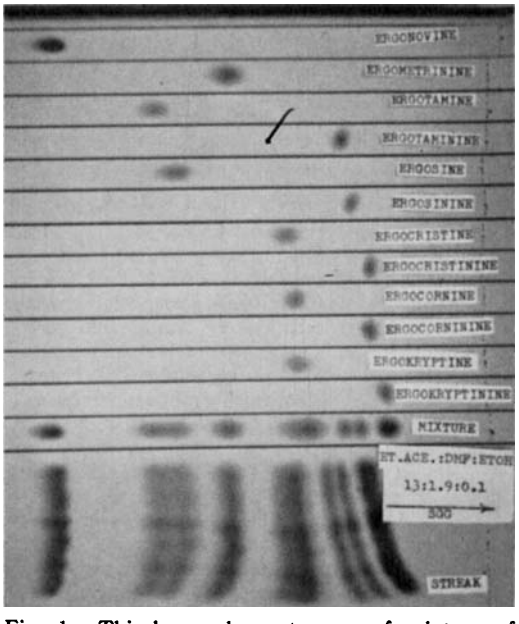

Fig. 1.—Thin-layer chromatogram of mixture of ergot alkaloids. (Solvent system *A*.)

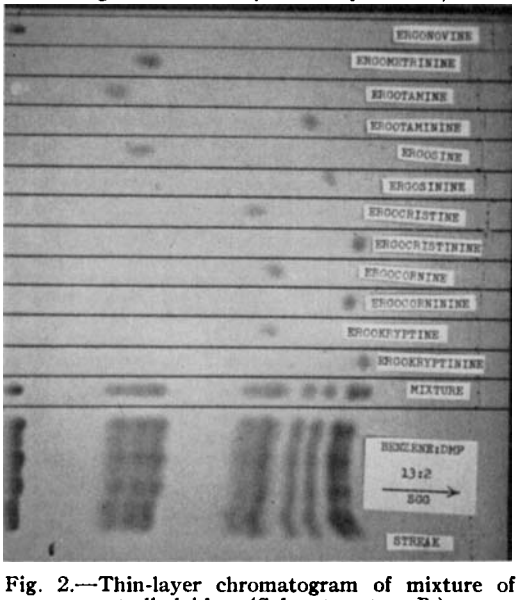

Fig. 2.—Thin-layer chromatogram of mixture of ergot alkaloids. (Solvent system *B*.)

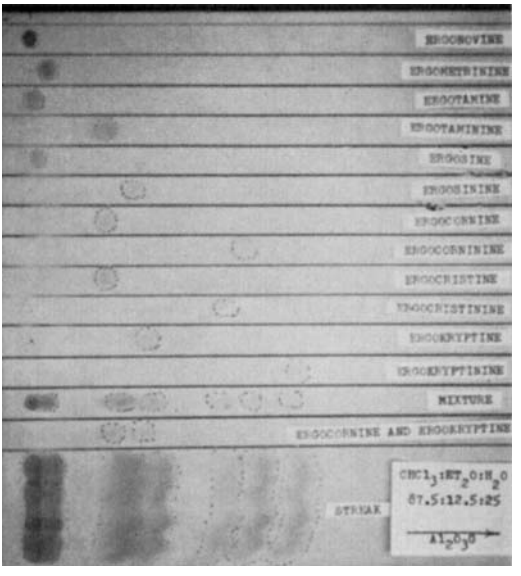

Fig. 3.—Thin-layer chromatogram of mixture of ergot alkaloids. (Solvent system *C*.)

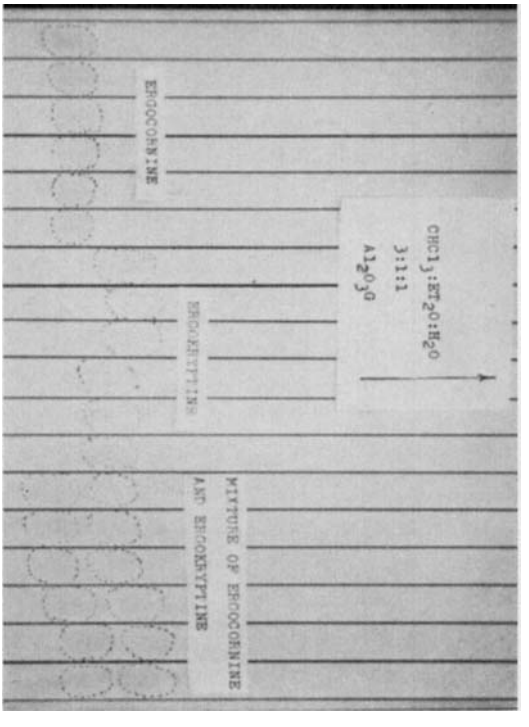

Fig. 4.—Thin-layer chromatogram of ergokryptine and ergocornine. (Solvent system *D*.)

Preparation of Developing Chambers.—Glass

TABLE I.—R_f VALUES OF ERGOT ALKALOIDS

Alkaloid[a]	Solvent System A on SGG		B on SGG		C Al_2O_3G		D Al_2O_3G	
	Mixture	Individually	Mixture	Individually	Mixture	Individually	Mixture	Individually
1	0.166 ± 0.008	0.173 ± 0.002	0.084 ± 0.002	0.120 ± 0.002	0.000	0.000	...	...
2	0.433 ± 0.013	0.441 ± 0.005	0.285 ± 0.012	0.384 ± 0.005	0.019 ± 0.002	0.008 ± 0.002	...	...
3	0.303 ± 0.008	0.313 ± 0.002	0.244 ± 0.011	0.308 ± 0.003	0.019 ± 0.002	0.010 ± 0.001	...	...
4	0.659 ± 0.012	0.682 ± 0.012	0.601 ± 0.023	0.638 ± 0.009	0.113 ± 0.010	0.065 ± 0.003	...	...
5	0.340 ± 0.009	0.350 ± 0.004	0.273 ± 0.012	0.314 ± 0.002	0.019 ± 0.002	0.016 ± 0.002	...	...
6	0.701 ± 0.011	0.747 ± 0.005	0.649 ± 0.022	0.677 ± 0.022	0.113 ± 0.010	0.115 ± 0.002	...	...
7	0.529 ± 0.009	0.544 ± 0.009	0.472 ± 0.017	0.558 ± 0.022	0.113 ± 0.010	0.093 ± 0.004	...	...
8	0.749 ± 0.013	0.796 ± 0.005	0.700 ± 0.023	0.743 ± 0.014	0.225 ± 0.016	0.379 ± 0.004	...	...
9	0.566 ± 0.011	0.579 ± 0.016	0.518 ± 0.019	0.588 ± 0.004	0.113 ± 0.010	0.095 ± 0.003	0.217 ± 0.017	0.243 ± 0.013
10	0.749 ± 0.013	0.833 ± 0.009	0.700 ± 0.023	0.726 ± 0.014	0.278 ± 0.020	0.310 ± 0.008	...	...
11	0.566 ± 0.011	0.598 ± 0.005	0.518 ± 0.019	0.554 ± 0.015	0.113 ± 0.010	0.148 ± 0.012	0.273 ± 0.019	0.299 ± 0.009
12	0.749 ± 0.013	0.854 ± 0.003	0.728 ± 0.021	0.746 ± 0.023	0.332 ± 0.028	0.457 ± 0.019	...	...

[a] *1*, Ergonovine; *2*, ergometrinine; *3*, ergotamine; *4*, ergotaminine; *5*, ergosine; *6*, ergosinine; *7*, ergocristine; *8*, ergocristinine; *9*, ergocornine; *10*, ergocorninine; *11*, ergokryptine; and *12*, ergokryptinine.

jars, 8¼ × 3 × 9 in. (internal dimensions) were lined with Whatman No. 1 filter paper as wicks. The solvent system (250 ml.) was added, the jars covered, leveled, and allowed to equilibrate for at least 30 minutes at 21 ± 0.5°.

Development of Chromatograms.—Plates were placed on a stainless steel support rack, immersed in the solvent pool, covered, and development allowed to proceed to within 1 cm. of the upper edge of the plate, unless otherwise specified. Ordinarily, development to this height required 80 minutes on SGG plates and 60 minutes on Al_2O_3 plates.

Detection of Alkaloids.—Short wavelength U.V. light and reaction with Ehrlich's reagent (5% *p*-dimethylaminobenzaldehyde in concentrated HCl) were used to locate the positions of alkaloids on the chromatograms.

Quantitative Determination of Alkaloids.—Solutions of alkaloids were assayed by mixing 1 part of the solution with 2 parts *p*-dimethylaminobenzaldehyde T.S. (U.S.P.), permitting reaction to occur for 30 minutes, and determining absorbance using a Beckman model DU spectrophotometer at a wavelength of 590 mμ with a 0.06-mm. slit width.

RESULTS AND DISCUSSION

Qualitative Analysis of Alkaloids.—Using solvent system *A* and SGG, the following alkaloids and mixtures of alkaloids (in order of increasing R_f values), were separated: ergonovine, ergotamine, ergosine, ergometrinine, ergocristine, ergocornine plus ergokryptine, ergotaminine, ergosinine, and a mixture of ergocristinine, ergocorninine, and ergokryptinine. Figure 1 shows a chromatogram of these results.

Using solvent system *B* and SGG, the same order of separation of the alkaloids occurred, with minor variations in resolution. The R_f values of ergometrinine and ergosine varied only slightly, but some resolution of ergokryptinine from ergocorninine and ergocristinine was achieved (Fig. 2).

With solvent system *C* and Al_2O_3G, poor resolution of the alkaloids occurred, except for ergocristinine, ergocorninine, and ergokryptinine. These three alkaloids were well separated from the other nine alkaloids and well separated from one another (Fig. 3).

Resolution of ergocornine from ergokryptine occurred in solvent system *C*, but separation could not be achieved in mixture with the other alkaloids because of the close proximity of ergotaminine, ergosinine, and ergocristine. Solvent system *D*, using Al_2O_3G, gave better resolution of ergocornine and ergokryptine, but again the separation was obscured by ergotaminine, ergosinine, and ergocristine.

Using either solvent system *A* or *B* and SGG, 50 μl. of the mixture of 12 alkaloids was applied to the

TABLE II.—SOLVENT SYSTEMS USED FOR RESOLUTION OF ALKALOIDS FOR QUANTITATIVE DETERMINATIONS

Alkaloid Combination	Solvent Systems
Ergonovine	*A* on SGG
Ergotamine + ergosine	*A* on SGG
Ergometrinine	*A* on SGG
Ergocristine + ergokryptine + ergocornine	*A* on SGG
Ergokryptine + ergocornine	*D* on Al_2O_3G
Ergotaminine + ergosinine	*A* on SGG
Ergocristinine + ergokryptinine + ergocorninine	*C* on Al_2O_3G

TABLE III.—STATISTICAL ANALYSIS OF THE ASSAYS[a]

Alkaloid	a ($\times 10^2$)	b ($\times 10^2$)	b^2 ($\times 10^6$)	n	$\bar{y}$	$\Sigma (x - \bar{x})^2$	s^2 ($\times 10^6$)
Ergonovine	−3.35	14.8	219.0	15	0.262	750	3.85
Ergometrinine	−5.26	14.8	219.0	10	0.243	500	9.63
Ergotamine	−2.40	7.92	62.72	10	0.134	500	3.86
Ergotaminine	−3.25	6.58	43.23	20	0.099	1000	7.77
Ergosine	−2.99	7.50	56.25	10	0.120	500	74.5
Ergosinine	−4.29	6.63	43.96	20	0.090	1000	15.8
Ergocristine	−3.36	7.83	61.31	25	0.123	1250	1.32
Ergocristinine	−3.45	7.31	53.33	25	0.117	1134	12.3
Ergocornine	−5.79	9.23	85.19	10	0.127	500	15.1
Ergocorninine	−1.76	4.73	22.40	25	0.081	1134	20.0
Ergokryptine	−5.40	7.56	57.18	10	0.098	500	13.9
Ergokryptinine	−2.31	6.17	38.08	24	0.104	1116	12.7
Ergocornine + ergokryptine	−5.77	7.93	62.82	25	0.259	5000	77.4

[a] y = Absorbance. x = Concentration (micrograms per milliliter). n = Total number of determinations. s^2 = Variance about the regression.

$$x = \frac{y - a}{b} \qquad \text{(Eq. 1)}$$

$$V(x) = \frac{s^2}{b^2}\left\{\left(\frac{1}{m} + \frac{1}{n}\right) + \left(\frac{Y - \bar{y}}{b}\right)^2 \frac{1}{\Sigma(x - \bar{x})^2}\right\} \qquad \text{(Eq. 2)}$$

where $V(x)$ = variance of a prediction of concentration from a new absorbance value, Y = absorbance of a new sample, and m = number of absorbance determinations of the new sample.

adsorbent in a line 7.5 cm. in length (Figs. 1 and 2). The chromatograms were examined under U.V. light, and the band corresponding to the mixture of ergocornine and ergokryptine was outlined. This area was scraped from the plate, the powder added to a test tube (9.5 × 1.5 cm.), and the alkaloids eluted with two 5-ml. portions of 10% methanol in chloroform. The eluates were filtered, combined in a 50-ml. round-bottomed flask, and the solvent removed under vacuum at 40°, using a Rinco model VE-1000B rotating evaporator.[5] Sufficient 10% methanol in chloroform was added to the flask to give a total volume of 50 μl.

This solution was spotted in the usual manner on Al_2O_3G, and the chromatogram developed with solvent system *D* (Fig. 4). Confirmation of the presence of one or both of these alkaloids was achieved in this manner.

Table I lists the average R_f values of the 12 ergot alkaloids in the various solvent systems. The R_f values of the alkaloids chromatographed as single compounds (individually) are the averages of six determinations. The R_f values determined when a mixture of all 12 alkaloids were chromatographed represent the averages of 36 determinations. Standard deviations were calculated in the usual manner. Reproducibility of the values differ significantly with each development. The order of separation and the resolution of the mixtures is constant, however.

Quantitative Analysis of Alkaloids.—With successful resolution for qualitative identification, the investigations were extended to studies of the resolution and quantitative estimations of 20 to 60 mcg. quantities of each of the 12 alkaloids. Because the ergot alkaloids are easily decomposed by light, the following manipulations were carried out in subdued light.

Solutions of the alkaloids were prepared in the following combinations: ergonovine; ergotamine and ergosine; ergometrinine; ergocristine, ergokryptine, and ergocornine; ergotaminine and ergosinine; and ergocristinine, ergokryptinine, and ergocorninine. Each solution contained 1 mcg./μl. of each alkaloid of the combination. Solutions were spotted on the adsorbents with a 10-μl. pipet to form a band as described previously. Each 10 μl. of solution was restricted in the length of spotting to 1.5 cm. on SGG and to 3.0 cm. on Al_2O_3G. After emptying the pipet, 10 μl. of 10% methanol in chloroform was drawn into it; this rinse was superimposed on the area just spotted. Using the pipet 20, 30, 40, 50, and 60-μl. quantities of the solutions were applied to the chromatograms as bands, varying in length from 3 to 9 cm. on SGG and 6 to 18 cm. on Al_2O_3G. The chromatograms were developed in the appropriate solvent system as shown in Table II. Chromatograms on SGG were developed to within 1 cm. of the top edge of the plate. Better resolution was obtained with chromatograms on Al_2O_3G by permitting continuous development for 120 minutes.

The chromatograms were examined under U.V. light and the bands outlined with a sharp stylus. The adsorbent within an outlined area was scraped from the plates with a razor blade onto glassine paper. The powder was then carefully transferred to a 9.5 × 1.5-cm. centrifuge tube; 2 ml. of a solvent composed of methanol:deionized water:glacial acetic acid (4.5:4.5:1.0) was added to the tube to affect desorption (3).

The solvent-adsorbent mixture was stirred with a Vortex Jr. mixer, model K-500J,[6] during an 8–10-minute period. Four milliliters of *p*-dimethylaminobenzaldehyde T.S. was added, the time noted immediately, and the mixture stirred with a glass stirring rod. The suspension was centrifuged at 4500 r.p.m. for approximately 7 minutes, and the supernatant was then decanted into a 1-cm. quartz spectrophotometer cell. Absorbance was determined 30 minutes after the addition of the *p*-dimethylaminobenzaldehyde T.S. as previously described.

The absorbance values of each alkaloid chromatographed were tabulated in terms of the original concentrations. These data were analyzed by the

[5] Rinco Instrument Co., Inc., Greenville, Ill.

[6] Scientific Industries, Inc., Queens Village, N. Y.

method of least squares, and the variance about the regression was determined (6). The statistical data are given so that concentration and variance for any given absorbance value may be determined by the equations in Table III.

Investigations were undertaken to study the effect on quantitative recovery of rechromatographing the ergokryptine-ergocornine mixture obtained by resolution with solvent system *A*, using solvent system *D* on Al_2O_3G. The earlier studies indicated that quantitative recovery of this mixture from chromatograms developed with solvent system *A* was possible. Quantitative recoveries of the separate alkaloids by chromatography of the mixture with solvent system *D* on Al_2O_3G were also successful. These data are presented in Table III.

Mixtures of several concentrations of ergocristine-ergokryptine-ergocornine were chromatographed with solvent system *A* in the usual manner. The bands corresponding to the ergokryptine-ergocornine mixture were removed, the alkaloids eluted, and the solution concentrated in the manner described previously. The residues were taken up in the appropriate volumes of 10% methanol in chloroform and applied to Al_2O_3G plates as bands. The chromatograms were developed with solvent system *D*. On examination of the chromatograms with U.V. light, several fluorescent bands were revealed. Two corresponded to the alkaloids chromatographed, two to isomers—ergokryptinine and ergocorninine—and at least three bands of unknown composition.

The bands corresponding to ergokryptine and ergocornine were outlined, the adsorbent removed, and the compounds eluted and analyzed in the usual manner. The absorbance values determined for replicate concentrations and between the several concentrations tested were too erratic to make the method useful.

CONCLUSIONS AND SUMMARY

1. The 12 ergot alkaloids investigated can be qualitatively identified in a mixture in concentrations of 2 mcg. by the thin-layer chromatographic procedures outlined.

2. Chromatography of the mixture on SGG using solvent system *A* resolves ergonovine, ergometrinine, ergotamine, ergotaminine, ergosine, ergosinine ergocristine, a mixture of ergokryptine and ergocornine, and a mixture of ergocristinine, ergokryptinine, and ergocorninine.

3. Ergokryptine and ergocornine can be identified by removing the mixture of them from SGG plates developed with solvent *A*, eluting the alkaloids from the adsorbent, and rechromatographing the eluate on Al_2O_3G with solvent system *D*.

4. Chromatography of the mixture on Al_2O_3G using solvent system *C* resolves ergocristinine, ergokryptinine, and ergocorninine from each other and the other alkaloids of the mixture.

5. All of the alkaloids, except for ergocornine and ergokryptine, can be resolved from a mixture of the 12 alkaloids and concentrations of 20 mcg. or more quantitatively recovered.

6. Quantitative recovery of a mixture of ergocornine and ergokryptine from chromatograms developed with solvent system *A* was demonstrated. The separate alkaloids can be resolved and quantitatively recovered from chromatograms developed with solvent system *D*.

7. The quantitative data have been analyzed statistically. The variance of the data about the regression is given so that the relative precision of the different assays is available. The other statistical parameters are presented so that the concentration and its variance may be determined when using the method as an assay procedure.

REFERENCES

(1) Teichert, K., Rochelmeyer, H., and Mutschler, E., *Deut. Apoth. Ztg.*, **100**, 283(1960).
(2) Klavehn, M., Rochelmeyer, H., and Seyfried, J., *ibid.*, **101**, 75(1961).
(3) Klavehn, M., and Rochelmeyer, H., *ibid.*, **101**, 477 (1961).
(4) Agurell, S., and Ramstad, E., *Lloydia*, **25**, 67(1962)
(5) Gröger, D., Tyler, V. E., Jr., and Dusenberry, J. E., *ibid.*, **24**, 97(1961).
(6) Davies, O. L., "Statistical Methods in Research and Production," 2nd ed., Oliver and Boyd, London 1949, pp. 118–167.