THE SEPARATION OF ALKALOIDS BY PAPER PARTITION CHROMATOGRAPHY

PART III. THE ASSAY OF ERGOT

BY J. E. CARLESS

From the Department of Pharmacy, University of Manchester

Received July 8, 1953

THE separation of certain water-insoluble alkaloids by partition paper chromatography has been described in a previous paper in this series[1], and this present communication describes an extension of the method to determine quantitatively both the water-insoluble and water-soluble alkaloids of ergot in the crude drug.

Up to the present time there have been no assay methods available for the determination of the individual alkaloids of ergot, with the exception of ergometrine which is referred to later. The physiologically active and inactive alkaloids and also some deterioration products such as the lysergic acids give the same colour with *p*-dimethylaminobenzaldehyde reagent used for the colorimetric assay in the British Pharmacopœia, 1948. This may be satisfactory for the estimation of total water-soluble and water-insoluble alkaloids, but does not give a true indication of the physiological activity. The object of this present paper was to show how the active and inactive alkaloids may be separated from each other by partitition chromatography and then assayed individually.

This is, of course, the ideal case and although some difficulties still remain, the author claims that the method in this paper is a considerable advance on previous assay methods. Ergots from different sources vary greatly in their alkaloidal content—both qualitatively and quantitatively; for instance Spanish and Russian ergots contain the ergotoxine group of alkaloids together with ergometrine, whilst Central European ergots are often rich in ergotamine[2]. The author's method of assay determines these individual alkaloids and also ergometrine and should be of importance to the manufacturer who wishes to purchase ergot for the preparation of ergotamine or ergometrine.

Chemical assay methods which have been devised for the physiologically active alkaloids in ergot have been limited to ergometrine, which is perhaps the most important ergot alkaloid. Grove[3] and Powell *et al*[4] devised methods by which the ergometrine was extracted and determined directly by colorimetric assay. Further collaborative work in the United States extending over several years has been published in a report on the assay of ergot issued by the American National Formulary Committee[5]. It is interesting to note that the specific assay for ergometrine devised by the American workers was finally not adopted for ergot itself owing to its complexity, and thus the final assay of the crude drug determines ergometrine together with ergometrinine. Foster *et al*[6], separated ergometrine in ergot-by paper chromatography and obtained semi-quantitative results, the error being approximately 20 per cent. Ergotamine has been separated

from its inactive isomer ergotaminine by counter current extraction[7], but this has not yet been applied to the crude drug. Circular[8],[9] and "reversed phase"[10], paper chromatographic methods for the qualitative separation of ergot alkaloids have been described.

For the quantitative determinations of individual alkaloids on filter paper chromatograms the alkaloidal spots have been extracted and assayed colorimetrically[1]. When the number of alkaloids in a mixture is small this may be satisfactory, but for more complex mixtures the methods becomes extremely tedious. Ether when used as a developing solvent for paper chromatograms involves difficulties such as maintaining saturated ether vapour in the chromatographic chamber, and incomplete saturation may lead to badly tailing spots and variable R_F values.

The extension of filter paper chromatography to columns was briefly discussed in a previous paper[1]. The column technique is more suitable for use with volatile solvents than is the filter paper strip method where saturation of the chromatographic chamber with vapour is difficult. In the following work buffered filter paper colums using Whatman ashless cellulose have been used throughout and with care give easily reproducible results.

Partition Chromatography of Pure Alkaloids—Qualitative

Details have been given for the separation of ergocristine and ergocristinine on buffered cellulose using Solka Floc cellulose[1]. Solka Floc is a finely-powdered cellulose and rate of solvent flow through it tends to be slow. In subsequent work Whatman ashless cellulose powder which became available was found to be more suitable.

For the preparation of columns 8 g. of Whatman ashless cellulose (standard grade) was mixed with 2 ml. of citrate-phosphate buffer (McIlvaine) *p*H 3·0 and packed dry into a glass tube of 1 cm. bore, 30 cm. long, into the lower end of which was fused a sintered glass filter (No. 3). The alkaloid in chloroform or ethanol solution was added to the column by first absorbing the solution on 0·3 g. of cellulose powder, evaporating off the solvent, and then mixing this powder with an equal weight of cellulose containing 40 per cent. water; the cellulose alkaloid mixture thus contained the same proportion of water as did the rest of the column. This powder was added to the top of the column, pressed down and covered with a layer of washed sand. The column was developed with anæsthetic ether saturated with water, and the alkaloids moved down the column in the following order—ergocristinine, ergocristine, ergosine and ergotamine; the water-soluble alkaloids ergometrinine and ergometrine remained stationary at the top of the column. From preliminary experiments it became apparent that by using a cellulose column buffered at *p*H 3·0 and ether as the developing solvent the slowly moving zones of ergosine and ergotamine would only be eluted by relatively large volumes of solvent. Raising the *p*H of the column resulted in more rapid movement of these zones, but in this case there was incomplete separation of the quickly moving ergocristinine and ergocristine. The difficulty was overcome by the addition of 0·1 per cent. of pyridine to the developing solvent, so that

as development of the chromatogram proceeded, the *p*H of the buffer on the column gradually increased from *p*H 3 to a value of *p*H 4·8 after the passage of 100 ml. of solvent. Under these conditions the slowly moving alkaloids were eluted more rapidly and the zones were considerably compacted. This may be considered as being analagous to gradient elution methods[11] since a *p*H gradient exists down the column. The eluate was collected in 2·5-ml. fractions by means of an automatic fraction collector, and each fraction was assayed colorimetrically using dimethylaminobenzaldehyde reagent. The first fraction collected always contained a considerable amount of cellulose extractive which gave a turbid solution on adding water and colour reagent. A slight amount of extractive also appeared in fractions 2 and 3, and these three solutions were filtered through No. 3 sintered glass filters for clarification.

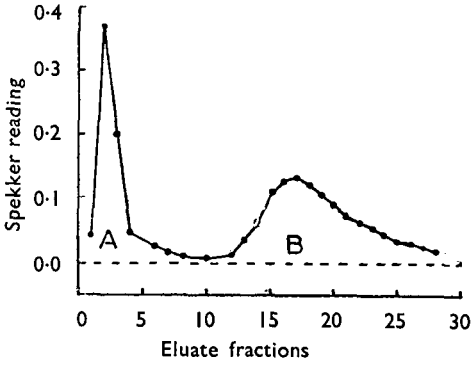

FIG. 1. Elution curve of ergocristinine (A) and ergocristine (B). Buffer *p*H 3·0. Anæsthetic ether saturated with water used as developing solvent.

The elution curves of ergocristinine and ergocristine when anæsthetic ether (saturated with water) was used are shown in Figure 1, and should be compared with Figure 2 where 0·1 per cent. of pyridine has been added to the developing solvent. The ergocristine was considerably compacted but tended to overlap the ergocristinine which appears in fraction 1 together with the cellulose extractive. To improve the separation the pyridine was not added until fraction 1 was collected. A better separation was thus achieved (as shown in Fig. 3) with the added advantage that the alkaloid did not break through until fraction 2, so that the cellulose extractive did not interfere. Figure 4 (*a*) shows typical elution curves for mixtures of pure alkaloids using this improved method. The amounts of alkaloids required for determination by colorimetric assay are so small that there is no danger of overloading the buffer on the column so that the shape and position of the elution curves are an indication of alkaloid identity. The positions of the alkaloidal peaks are summarised in Table IV. The slight variation in the position of the peaks is probably due to differences in packing the column, although this was standardised as far as possible. During hot weather increased evaporation of the eluate from the outlet of the column will also be another source of variation.

After the passage through the column of 100 ml. of anæsthetic ether (saturated with water) containing 0·1 per cent. of pyridine the *p*H of the column had risen from the original value of *p*H 3 to *p*H 4·8, and the water-insoluble alkaloids were completely eluted. The water-soluble alkaloids remained at the top of the column and were later eluted by making the column alkaline, when a complete separation of ergometrine and ergo-

metrinine was obtained. The column was made alkaline by passing through 10 ml. of developing solvent containing 0·1 ml. of diethylamine, and development was continued with anæsthetic ether saturated with water. Figure 4 (*b*) shows a typical elution curve of the water-soluble alkaloids after the elution of the water-insoluble alkaloids.

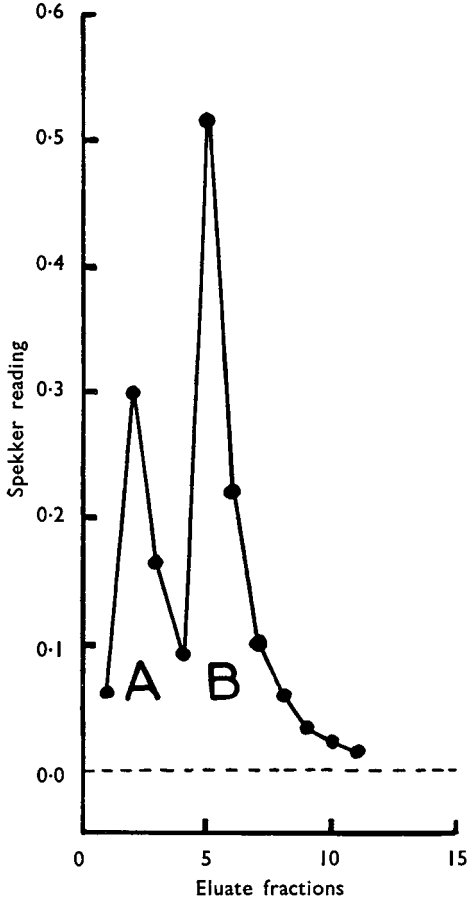

FIG. 2. Elution curve of ergocristinine (A) and ergocristine (B). Buffer *p*H 3·0. 0·1 per cent. pyridine added to developing solvent.

Absorptiometric Assay of the Fractions. Assays were carried out on the individual 2·5 ml. fractions after the solvent had evaporated off at room temperature. In some cases low recovery figures were observed especially when the alkaloidal residues had dried overnight. The fractions were collected in 50-ml. Pyrex beakers and a considerable residue of undissolved alkaloid was apparent on the sides of the beakers, even after the development of the colour reaction. This was finally overcome by the addition of a small volume of lactic acid to each beaker before collecting the fractions, the acid dissolved in the ether fraction and on evaporation of the ether a thin film of lactic acid and alkaloidal lactate was left, which was readily soluble in 1 per cent. tartaric acid solution or water, before the addition of the dimethylaminobenzaldehyde reagent. The lactic acid was most conveniently added to the beakers in the form of an ethereal solution—1 ml. of 5 per cent. solution in anæsthetic ether was added to each beaker. The volume of water or tartaric acid solution used was 1·95 ml., and 4 ml. of dimethylaminobenzaldehyde reagent was added. The developed colour was measured in the usual way in a Spekker absorptiometer using 1 cm. path cell and filters with maximum transmission at 590 mμ.

PARTITION CHROMATOGRAPHY OF PURE ALKALOIDS—QUANTITATIVE

Solutions of pure alkaloids in ethanol (50 to 80 per cent.) were freshly prepared and assayed absorptiometrically after dilution with 1 per cent.

TABLE I

SEPARATION OF ERGOT ALKALOIDS ON BUFFERED CELLULOSE COLUMNS

(Alkaloid calculated as ergotoxine and expressed in μg.)

Ergocristinine		Ergocristine		Ergosine		Ergotamine		Ergometrinine		Ergometrine	
Taken	Found	Taken	Found	Taken	Found	Taken	Found	Taken	Found	Taken	Found
	13	247	229								
	15	247	234								
366	370	296	264								
296	266	234	206	152	138						
										180	180
						88	72			270	235
								95	84	135	141
						262	207				
						262	215				

TABLE II

EXTRACTION OF ALKALOIDS FROM ERGOT BY MICROPERCOLATION WITH VARYING VOLUMES OF CHLOROFORM (5 per cent. ETHANOL)

Weight of ergot g.	Vol. of percolate ml. *	Alkaloid found (calculated as ergotoxine) per cent.	Time of percolation
0·5168	25	0·324	4½ hours
0·5022	25	0·329	1½ hours
0·5780	30	0·320	overnight
0·5030	10	0·338	1½ hours
0·5180	5	0·319	¾ hour
0·5154	5	0·320	1 hour

* Percolate evaporated, if necessary, to 10 ml., 50 ml. of anæsthetic ether and 5 ml. of acetone added and extracted with 4 quantities, each of 10 ml., of 1 per cent. sulphuric acid; mixed acid solutions made up to 50 ml. and assayed colorimetrically.

TABLE III

COMPARISON OF B.P. METHOD AND MICROPERCOLATION METHOD FOR THE TOTAL ALKALOIDS OF ERGOT

Sample of Ergot	Total Alkaloids expressed as Ergotoxine	
	B.P. Assay per cent.	Micropercolation* per cent.
AW	0·320	0·320
B	0·157	0·168
C	0·255	0·231
E	0·442	0·476
F	0·169	0·163
H	0·390	
JW	0·346	0·351

* Ergot percolated with 6 ml. of chloroform (5 per cent. of ethanol).

tartaric acid solution. Suitable volumes of the solutions (usually 0·3 to 1·0 ml.) were added to the column by first absorbing on cellulose as described previously. The dish containing the alkaloid cellulose mixture was carefully cleaned out with washed sand which was added to the column, and was also rinsed out with some of the developing solvent.

Water-insoluble Alkaloids. Column—8 g. of cellulose plus 2 ml. of citrate-phosphate buffer *p*H 3·0; developing solvent—anæsthetic ether saturated with water. 0·1 per cent. of pyridine A.R. was added after fraction 1 collected. Assay of the fractions—as described in previous section.

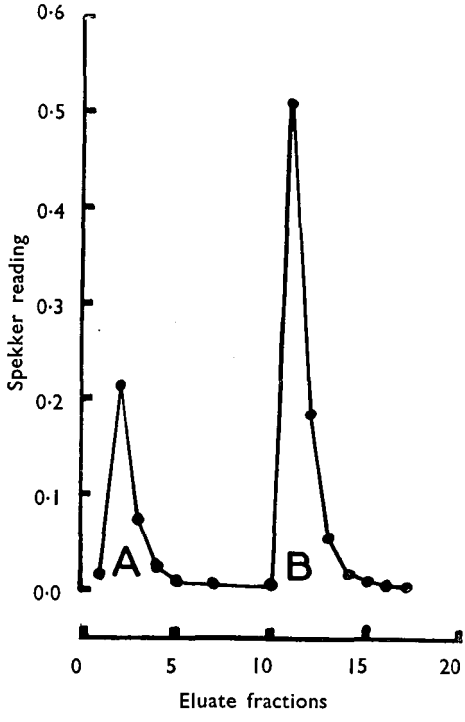

FIG. 3. Elution curve of ergocristinine (A) and ergocristine (B). Buffer *p*H 3·0. 0·1 per cent. pyridine added to developing solvent after fraction 1 collected.

Water-soluble Alkaloids. Developing solvent—0·1 ml. of diethylamine in 10 ml. of water-saturated ether was passed through the column and then development continued with water-saturated ether. 18 ml. of eluate was collected and discarded before the column was connected to the automatic fraction collector. In most cases 10 per cent. of ethanol was used to speed up the elution of ergometrine. (*See* individual graphs.)

The quantitative recoveries are shown in Table II. The average recoveries of the alkaloids with the exception of ergotamine, were about 90 to 95 per cent. The low recovery of ergotamine may be due to its slow elution from the column and the recovery figure is the summation of about eighteen assays. A total volume of 120 ml. of solvent was used to develop the column and 40 fractions collected. The rest of the eluate was collected as a separate fraction and was usually found to contain a small amount of ergotamine, which was included in the recovery figure. Lysergic and *iso*lysergic acids do not interfere with this assay since they remain at the top of the column after the elution of the total alkaloids. Extracted colouring matter from the ergot also remains at the top of the column.

THE ASSAY OF ERGOT

The previous part of this paper gives the method adopted for separations of the pure alkaloids, and in order to apply this to ergot itself it is necessary

to consider the following operations. 1. Extraction of the alkaloids from the drug. 2. Transfer of the alkaloids to the chromatographic column. 3. Partitition chromatography of the alkaloids.

Extraction of Ergot. The B.P. method of continuous extraction with ether was not considered suitable since hydrolysis and interconversion of of the alkaloids may occur in hot alkaline ether. Only semimicro quantities of ergot (0·5 to 1·0 g.) are required for the chromatographic assay and a rapid cold extraction method was devised. The alkaloids were liberated by mixing a solution of sodium bicarbonate with the powdered ergot, which was then ground thoroughly with sand before percolation with chloroform containing 5 per cent. of ethanol. Chloroform has been found to give the highest assay figures for total and water-soluble alkaloids.[5] The details of the extraction method are as follows. About 0·5 g. of defatted ergot was accurately weighed and mixed thoroughly with 0·2 ml. of water and 0·03 g. of sodium bicarbonate in a small mortar. 1 g. of neutral acid-washed sand was added and the mixture ground for a few minutes. The powder was transferred to a micropercolator which consisted of a glass tube 10 cm. long, 1 cm. bore and closed near one end with a No. 2 sintered glass filter (a Pyrex micro filter SF. 8 was found very suitable.) The powder was added to the percolator in 3 approximately equal amounts and pressed firmly down after each addition with a glass rod. The mortar was cleaned out with 2 successive 1 g. amounts of sand to remove any residual ergot and the sand placed in the percolator. The drug was then percolated with chloroform to which 5 per cent. by volume of ethanol had been added, and was found to be exhausted after the collection of 5 to 6 ml. of percolate (*see* Table II). The rate of percolation was approximately 1 drop every 1 to 3 seconds. A comparison of this micro method with the B.P. method of extraction is shown in Table III and a good agreement between the two methods was obtained. The micro percolation method was thus considered a suitable extraction process for the total alkaloids.

TABLE IV

Alkaloid	Position of maximum on elution curve Fraction number
Ergotinine	2 or 3
Ergotoxine	11 – 13
Ergosinine	11 – 13
Ergosine	18 – 21
Ergotaminine	11 – 13
Ergotamine	26 – 29

Transfer of the Alkaloids to the Chromatographic Column. This was most conveniently done by absorbing the chloroform as it dripped from the percolator on warmed cellulose and this was later transferred to the column. The details are as follows.

0·3 g. of cellulose in a small Pyrex evaporating dish was warmed to 50° to 60° C. and the micropercolator containing the ergot was mounted above it, the outlet tip practically touching the cellulose. 6 to 8 ml. of chloroform (5 per cent. ethanol) was added to the percolator and, towards the end of the percolation, slight positive pressure was applied to displace the solvent. To ensure rapid evaporation of the chloroform a gentle current of air was passed over the dish. After the evaporation of the

chloroform an equal weight of cellulose containing 40 per cent. water was added and well mixed with a glass rod. The powder was transferred to the top of the column and the dish cleaned with two successive 1 g. amounts of sand which was transferred to the column. The dish was also rinsed out with some of the developing solvent.

TABLE V

COMPARISON OF THE PARTITION CHROMATOGRAPHIC METHOD AND THE B.P. ASSAY

	Chromatographic Assay								B.P. Assay	
	Water-insoluble Alkaloids				Water-soluble Alkaloids		Total water-insoluble Alkaloids	Total water-soluble Alkaloids		
Sample of Ergot	Ergotinine A	Ergotoxine B	Ergosine C	Ergotamine D	Ergometrinine E	Ergometrine F			Water insoluble	Water soluble
AW	24·8 19	125 125	37·7 45·1	— —	14·7 13	20 20·3	188 189	34·7 33·3	233	46·8
B	9·8 7·7	59·1 68·5	15·1 20·8	11·5 13·5	6·2 5·2	11·1 9·4	96 111	17·3 14·6	131	14·0
C	35·4	93	20	29·8	2·7	16·4	178	19·1	223	18·8
E	23	177	38	53	18·5	38	301	56·5	330	60·2
F	8·7	53·3	7	44·6	4·2 5·2	2·4 3·4	114	6·6	144	13·5
H	26·7	147	62·3	—	13·7	22·8	236	36·5	260	70
JW	23	173	72	—	17·3 15	35·4 31	268	52·7 47	261	45·8

Water-insoluble alkaloids calculated as ergotoxine and expressed in mg./100 g. of defatted drug.
Water-soluble alkaloids calculated as ergometrine and expressed in mg./100 g. of defatted drug.

Partition Chromatography of the Alkaloids. The method used was the same as for the quantitative separation of the pure alkaloids which has been discussed in an earlier part of this paper. The position of the peak of the elution curve for each of the alkaloids was found to be the same as in the case of the pure alkaloids, and provided a means of identification.

TABLE VI

GEOGRAPHICAL SOURCE OF THE ERGOT SAMPLES ASSAYED

Sample	Geographical Source
AW	Spain (Supplied in 1946 by the wholesaler, as defatted ergot)
B	Odenwald, Germany. 1951 crop
C	Austria
E	Alsace. 1951 crop
F	Black Forest, Germany. 1951 crop
H	Portugal. 1950 crop
JW	Spain or Portugal (Supplied in 1952 by the wholesaler, as defatted ergot)

Samples B, C, E, F, H, were kindly supplied by Professor A. Stoll.
All samples were supplied as being *Claviceps purpurea* grown on rye.

Identification by other methods, such as by melting point determinations or by preparation of crystalline derivatives is difficult. Identification of an unknown peak was also checked in most cases by the addition of the pure suspected alkaloid to the ergot extract and noting the position of the

increased peak. Further confirmation of identity was also carried out by paper chromatography on buffered filter paper[1] and on unbuffered paper[6] for the water-insoluble and water-soluble alkaloids respectively.

Fat which was always found in ergots defatted with light petroleum by the B.P. method appeared in fractions 1, 2 and 3. Fraction 1, which should be alkaloid-free, contained most of the fat and was not assayed. The

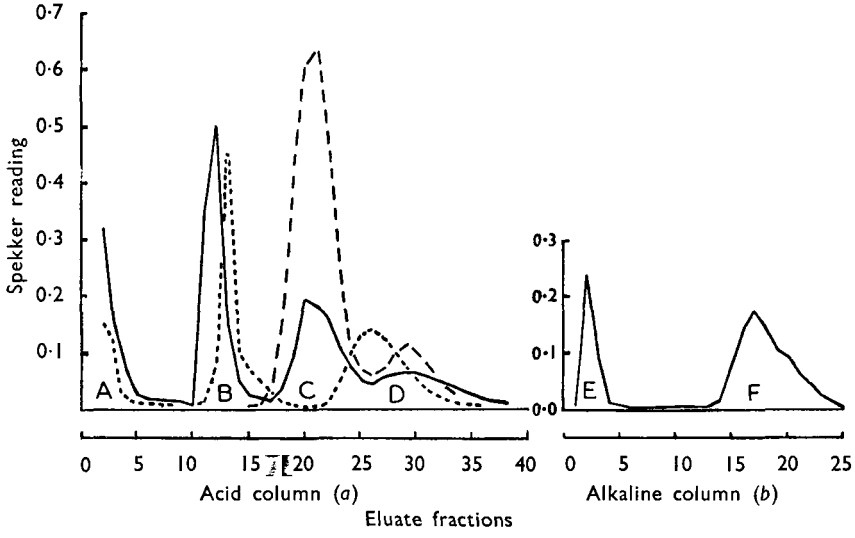

FIG. 4*a*. Elution curves of the water insoluble alkaloids on buffered column *p*H 3·0. 0·1 per cent. pyridine added after fraction 1 collected.

	A. Ergocristinine.	B. Ergocristine.	C. Ergosine.	D. Ergotamine.
————	0·15 mg. A.	0·3 mg. B.	0·25 mg. C.	0·14 mg. D.
- - - - -	0·07 mg. A.	0·25 mg. B.		0·25 mg. D.
— — —			0·8 mg. C.	0·2 mg. D.

FIG. 4*b*. Elution curve of the water soluble alkaloids on buffered column (original *p*H 3·0) made alkaline by the passage of 0·1 ml. diethylamine in 10 ml. of developing solvent.

E. Ergometrinine 0·05 mg. F. Ergometrine 0·14 mg.

Fraction 1 collected when ergometrinine 4 cm. from base of column.

estimation of alkaloid in fractions 2 and 3 was slightly modified in view of the fat content. 3·95 ml. of 1 per cent. tartaric acid solution was added to the alkaloid and fat residue, the mixture warmed to 60° C. for a few minutes, stirred, cooled and then filtered through Whatman No. 1 paper. 2 ml. of the clear filtrate was then assayed. (On the accompanying graphs, to keep the scale of the elution curves unchanged the Spekker readings for fractions 2 and 3 have been converted to values which would have been found if the normal value of 1·95 ml. of tartaric acid solution had been added.)

The geographical source of the ergots examined is shown in Table VI and the elution alkaloid curves for three samples are shown in the Figures 5 to 7. The alkaloidal content is summarised in Table V and these assay figures have been obtained by the summation of the individual colour

assays. Where incomplete separation of the two zones has occurred an arbitrary division into two zones has been made at the point of inflexion of the curve.

It is not claimed that all the active alkaloids are separated completely from the inactive ones by using this chromatographic method since the ergotoxine and ergosine fractions may contain some ergosinine and

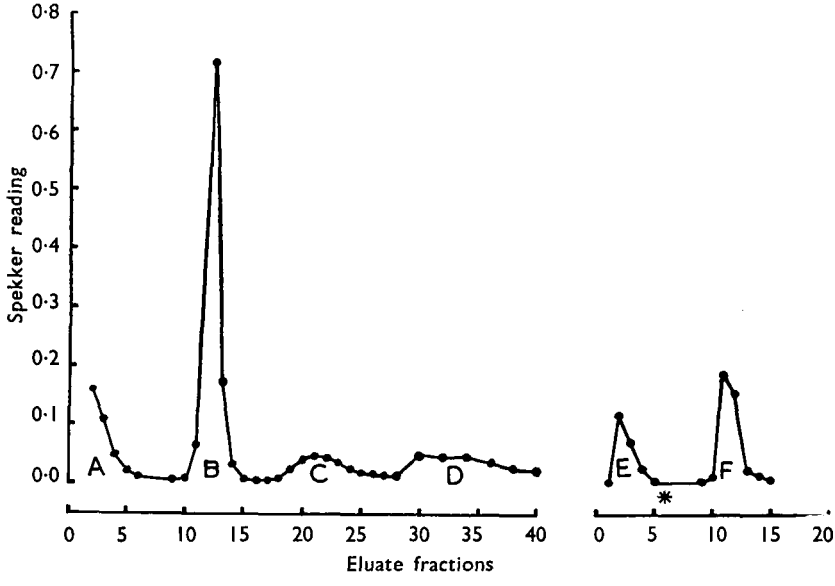

FIG. 5. Elution curves of the alkaloids of ergot. Sample B (German).
* 10 per cent. of ethanol added.

ergotaminine and it will be necessary to chromatograph these fractions again using a different solvent system in order to obtain more information about their relative proportion. The separation of ergometrinine from ergometrine is much more distinct.

The British Pharmacopœia assay has been criticised by Foster *et al.*[6] since it gave high figures for water-soluble alkaloids when compared with the U.S. National Formulary assay and a paper chromatographic assay method described by them. The high figure will be partly due to ergometrinine being estimated as ergometrine, but in addition it was suggested that hydrolysis of the alkaloids to the lysergic acids during the B.P. extraction resulted in these being estimated as water-soluble alkaloids. The chromatographic assay of the present author excludes the lysergic acids since these are retained on the column. A comparison of the chromatographic and B.P. assays of 7 samples of ergot is shown in Table V. The B.P. assay figures for total water-soluble alkaloids in ergots AW, F and H were considerably higher than those found by the chromatographic method, but there was reasonable agreement between the assays of the remaining samples. The inactive ergometrinine constituted about one-third of the total water-insoluble alkaloids. In all the B.P. assays the

extraction flask was heated on a water bath, the ether level being well above the heating surface, and the flask was covered with cotton wool to exclude light. If the extraction flask is deeply immersed in a hot water bath then any alkaloid deposited on the sides of the flask due to loss of solvent, may be considerably overheated. Such overheating was found to

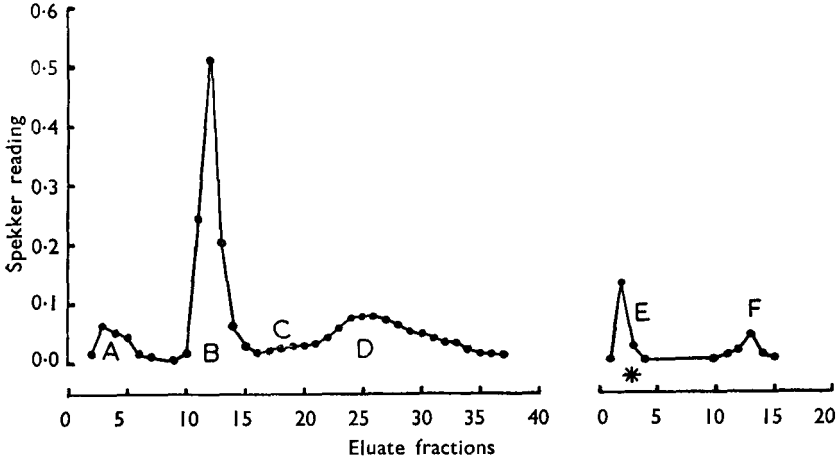

FIG. 6. Elution curves of the alkaloids of ergot. Sample F (German).
* 10 per cent. of ethanol added.

give a high figure for water-soluble alkaloid presumably due to decomposition. The adverse effect of exposing alkaline solutions of ergot alkaloids to bright daylight has been emphasised by the N.F. Committee[5]. During the B.P. assays alkaline solution of ergot alkaloids were exposed only to diffused daylight and the transfer of the alkaloids to the acid solution was

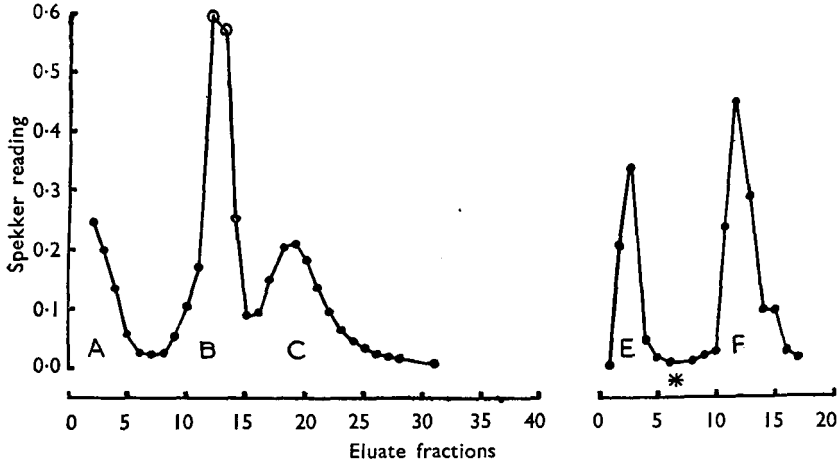

FIG. 7. Elution curves of the alkaloids of ergot. Sample JW (Spanish).
⊙ 3·95 ml. of water and 8 ml. of colour reagent.
* 10 per cent. of ethanol added.

carried out as quickly as possible. It appears advisable that the B.P. should include the above-mentioned precautions in the directions for the official assay to ensure uniformity.

During the examination of an alkaline hydrolysate of ergotamine in methanol, the column partition chromatogram revealed a zone at the same position that ergometrinine occupies. This could not be due to the lysergic acids since these remain at the top of the column. The presence of ergine (the amide of lysergic acid) was suspected since this has been prepared by alkaline hydrolysis of ergotamine and ergotoxine in methanol solution[12]. This was later confirmed by chromatographing a sample of pure ergine. Paper chromatography of the ergometrine and ergometrinine eluate fractions from the ergot samples using Whatman No. 1 paper with butanol-acetic acid-water[6], gave only the characteristic spots of ergometrine and ergometrinine. Ergine which moves more slowly than the water-soluble alkaloids in this solvent system was not detected. Thus there was no evidence of ergine being present in the original ergots, nor of its being produced during the chromatographic process.

Discussion

The separation of the water-soluble alkaloids from the water-insoluble alkaloids before colorimetric assay of these two groups, has been the basis of most of the published assay methods of ergot. The determination of the individual alkaloids within each group is complicated by their closely related chemical and physical properties. Partition chromatography offers a partial solution to these difficulties, and alkaloids whose pK_b and/or solubility values differ slightly from one another may be completely separated on buffered columns. Thus the *l*-and *d*-pairs of the ergot alkaloids may be separated from each other, the physiologically inactive *d*-form moving faster than the *l*-form. The problem is not so easy when several alkaloids are present together since the *l*-form of one alkaloid may overlap the *d*-form of another alkaloid; for instance, ergotoxine and ergotaminine are not separated in the system used. In such cases it is necessary to run additional chromatograms on paper to check the purity of the fraction.

Alkaline solutions of ergot alkaloids, especially if exposed to light undergo rapid decomposition[5]. Acid aqueous solutions are much more stable. The conditions under which the alkaloids are separated in this chromatographic method are unlikely to result in their decomposition, since the water-insoluble alkaloids are separated under acid conditions (*p*H 3 to 5) and the water-soluble alkaloids are only in alkaline solution for a relatively short time, during their passage down the column, the fractions being acidified on collection, with lactic acid. The chromatographic method also has the added advantage that the bulk of the alkaloids are protected from light, so that beyond covering the chromatographic apparatus to exclude bright daylight no other precautions were taken. In the recovery experiments using pure alkaloids 90 to 95 per cent. of the original alkaloid added was recovered, with the exception of ergotamine of which about 80 per cent. was recovered. This low figure may be

partially due to the wide spread of the elution curve and might be improved by bulking together the fractions. This has not been adopted so far since the shape of the elution curve has been used to predict the identity of the alkaloid. Recoveries of the water-soluble alkaloids appear to be a little higher than those of the water-insoluble alkaloids and this may be due to the increased purity of the cellulose after the passage of a large volume of solvent. It should not prove difficult to simplify this method for routine assay work if the determination of the individual water-insoluble alkaloids is not required. The first 5 to 10 ml. of eluate containing the inactive ergotinine could be discarded, and the next 120 to 150 ml. containing the water-insoluble alkaloids collected, the alkaloid being shaken out into acid solution and assayed. The water-soluble alkaloids could then be collected in fractions, either by means of a fraction collector or guided by inspection of the column under ultra-violet light to show up the position of the fluorescent alkaloid.

This chromatographic method offers many advantages over the present methods of evaluating ergot. Only a small sample is necessary and the assay can be completed within 2 days. The practical uses are not confined to the manufacturer who wishes to determine the proportion of individual alkaloids, but the method should be of value in assessing the physiological activity of the drug itself.

SUMMARY

1. A method for separating the water-insoluble alkaloids ergotinine, ergotoxine, ergosine, ergotamine and the water-soluble alkaloids ergometrine and ergometrinine by partition chromatography on buffered cellulose columns has been described.

2. The recoveries of pure individual alkaloids were consistent, being about 90 per cent., with the exception of ergotamine of which about 80 per cent. was recovered.

3. The method has been applied successfully to defatted ergot, of which only 0·5 g. was required for the assay.

4. 7 samples of ergot were assayed by the partition chromatographic method and the British Pharmacopœia, 1948, assay method and a comparison of the two methods was made.

5. 3 samples of Spanish Portugese ergots were found to contain ergotinine, ergotoxine, ergosine, ergometrine and ergometrinine. Ergotamine was not detected. 4 samples of Central European ergots differed from the Spanish ergots since they contained ergotamine and generally smaller amounts of ergosine.

In conclusion I wish to express my thanks, to Professor A. Stoll for supplying samples of commercial ergots, ergotamine tartrate, ergosine, ergosinine, and ergine, to Dr. G. E. Foster for supplying samples of ergotoxine, ergosine and ergosinine and to Professor H. Brindle, under whose direction this work has been carried out.

REFERENCES

1. Brindle, Carless and Woodhead, *J. Pharm. Pharmacol.*, 1951, **3**, 793.
2. *British Pharmaceutical Codex*, 1949.

3. Grove, *J. Amer. pharm. Ass. Sci. Ed.*, 1941, **30**, 260.
4. Powell, Reagan, Stevens and Swanson, *ibid.*, 1941, **30**, 255.
5. *Bull. Nat. Form. Comm.*, 1948, **16**, 1.
6. Foster, Macdonald and Jones, *J. Pharm. Pharmacol.*, 1949, **1**, 802.
7. Hellburg, *Farm. Revy.*, 1951, **50**, 17.
8. Berg., *Pharm. Weekbl.*, 1951, **86**, 900.
9. Berg., *ibid.*, 1951, **87**, 282.
10. Tyler and Schwarting. *J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 354.
11. Alan and Williams, *Ark. Kemi. Min. Geol.* 1951, through Endeavour **1952, 11, 5.**
12. Smith and Timmis, *J. chem. Soc.*, 1932, 763, 1543.

DISCUSSION

The paper was presented by MR. J. E. CARLESS.

DR. G. E. FOSTER (Dartford) said that to carry out a large number of colorimetric estimations would be rather time-consuming, and he asked whether, if it became a general routine method, some electronic device could not be constructed for carrying out the assay. There was no doubt that ergotoxine and ergotamine could co-exist in the same ergot. With regard to nomenclature, he pointed out that the authors referred to ergotinine and ergocristine, and it would be better to use the same name for the alkaloid. In the case of the water-soluble alkaloids, he noticed that the authors raised the *p*H in order to elute them from the cellulose, and he wondered whether they had considered the possibility of ergometrine being converted into ergometrinine.

MR. H. B. HEATH (Sudbury) said it was interesting to note that the N.F.IX used filter 590 mμ for ergot, whereas the U.S.P. XIV filtered at 540 to 560 mμ. He desired to know whether Beer's Law was more accurately followed at 550 than at 590 mμ. He had found no difference in peaks between ergometrine or ergotoxine in tartaric acid solution as obtained in an assay of ordinary ergot.

DR. W. MITCHELL (London) asked whether the authors had any information as to the relative stability of the alkaloids in different ergots. He had some reason to believe that the stability of alkaloids in liquid extract of ergot B.P. varied with different batches of ergot.

PROFESSOR H. BRINDLE (Manchester) said that he and his co-workers were comparatively satisfied with the assay of ergot after some 5 or 6 years intensive work. The apparatus used for the assay included a mechanical device which moved when a certain amount of eluate had been collected, and prepared for the next portion of eluate. It was not time-consuming at all.

MR. J. E. CARLESS, in reply, referring to the time taken in assays said that the initial extraction of the defatted ergot, could be completed in about 1 hour, the transfer of the alkaloids to the column was easily carried out, and then the use of a siphon escapement mechanism made the fractionation automatic. He had considered the use of radio frequency methods for detecting the presence of alkaloids in the eluate as it came from the column. He admitted that the terminology he had used was not as clear as it might be. Where he referred to the ergotoxine group of alkaloids this included ergocornine, ergocristine and ergokryptine. Where ergocristine was mentioned it was a pure sample of ergocristine

which had been used and, similarly, with ergocristinine. There was no separation of the individual members of the ergotoxine group or the ergotinine group. The conversion of active ergometrine into inactive ergometrinine was a possibility, especially if the alkaline solution of ergometrine was stored for any length of time, but there was no evidence of that having occurred on the column. He had followed the N.F. recommendation of using filters of 590 mμ which gave a straight line calibration curve up to about 0·006 or 0·008. After that it tended to diverge. He had little information concerning the stability of the alkaloids in different ergots but from earlier work he had concluded that liquid preparations of ergot were most stable at *p*H 3. While Spanish and Portugese ergots showed little difference in alkaloidal content, Central European ergots varied considerably from batch to batch.